International Journal on Immunorehabilitation

Volume 20 No 2
2018

Moscow

"Meditsina–Zdorov'e" Publishers

ISSN 1562-3629

INTERNATIONAL JOURNAL ON IMMUNOREHABILITATION
МЕЖДУНАРОДНЫЙ ЖУРНАЛ ПО ИММУНОРЕАБИЛИТАЦИИ

Official Journal of the World Immunopathology Organization

Volume 20 No 2
2018

International Journal on Immunorehabilitation
is indexed in the following international editions:
Excerpta Medica
Immunology Abstracts
Science Citation Index

The volume is sent to be indexed in Conference Proceedings
Citation Index by Thomson Reuters - Philadelphia formerly ISI

International Journal on Immunorehabilitation is registered by the Russian Federation State Press Committee. Registration No 019131.

EDITORIAL OFFICE

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Web site: www.isir.ru

ISSN 1562-3629

International Journal on Immunorehabilitation

Международный журнал по иммунореабилитации

Volume 20 No 2 **2018**

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ISSN 1562-3629

International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

The Alberto Oehling Keynote Lecture

EFFECT OF MODERNIZATION ON THE DEVELOPMENT OF ALLERGIES AND ASTHMA

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Industrialization and socioeconomic developments have been associated with modernization of lifestyle and subsequent changes in human health and disease. This presentation summarizes the impact of modernization on the development of allergies and asthma regarding the change in prevalence, the responsible causes, and the possible underlying mechanism.

Key words: *allergy, asthma, prevalence.*

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UDC 614.4, 61.616

RECENT TRENDS IN PREVALENCE OF ALLERGIES AND ASTHMA

Whereas many infectious diseases have declined during recent decades, several studies have shown a consistent global increase in allergic disorders. The increase was not only in the prevalence but also in manifestations, severity, earlier age of onset, and multiplicity of causative allergens. The impact was first evident in the respiratory manifestations and later on the cutaneous, gastrointestinal, food allergies, and systemic anaphylaxis. The increase in asthma prevalence began in North America, Western Europe, Japan, and Australia, then in countries undergoing industrialization and areas moving towards urbanization.

The rising trend of allergies and asthma is confirmed by data derived from studies showing:

- More prevalence in urban than in rural populations.
- More in recent generations of the same population.
- Correlation with the socioeconomic level.
- More in migrants from developing countries after livingin industrialized ones.
- Asthma has been an increasing cause for military service exclusion

CAUSES OF THE INCREASING PREVALENCE

Apart from the major role of genetics, numerous environmental and behavioral factors are involved directly (allergen exposure) and indirectly (contributory factors).

A. Increasing Allergen Exposure

FOOD:

- Infant feeding; baby formulas > breast milk
- Food consumption in quantity and variety (obesity "pandemic"), including highly allergenic foods: peanut, tree nuts, fish, milk, egg, soy.
- Eating out; "Buffet restaurants"; "All you can eat".
- Cross-reactivities between foods and with non-ingestant allergens, e.g. latex, mite, cockroach, pollen.
- Commercial foods incorporate multiple nutrient & non-nutrient allergenic ingredients.
- Incorporation of food protein in diagnostic & therapeutic agents, particularly dermatologic.
- Alcoholic drinks contain many natural allergens and added extrinsic allergens.

INDOOR ALLERGENS:

- HD mite, mold, pets
- Cosmetics, hair dyes, hair spray, acrylic nails
- Jewelry
- Tattoos and body piercing

OUTDOOR ALLERGENS:

- Mold, animal hair
- Occupational allergens

INDUSTRIAL JOBS:

- Inhalant allergens
- Contactant allergens

MEDICATIONS:

- Synthetics
- Biologicals
- Herbals

B. Increasing Contributory Factors

WESTERN LIFESTYLE:

- Indoor living (house allergens and irritants, vitamin D deficiency).
- Stress (work, family)
- Smaller family size
- Obesity
- Social events: allergenic foods, alcoholic drinks
- Longevity perpetuates the genetic trait

MEDICAL PRACTICES:

- Control of infectious diseases
- Caesarean section deliveries
- Over-prescribing antibiotics
- Imbalance of body microbiota
- Widespread use of acetaminophen/paracetamol
- Overuse of antacids
- Popularity of infant multivitamins
- Over-prescription of multiple medications
- Latex gloves and products
- Longer life expectancy: Longer interaction between genetic predisposition and environment. It also perpetuates the genetic atopic trait.

INDOOR AIR POLLUTANTS:

- Household cleaning products, tobacco smoke, radon, carbon monoxide, carbon dioxide, volatile organic compounds.

OUTDOOR AIR POLLUTANTS:

Automotives, factories, tobacco smoke, and natural disasters emit large quantities of particulate matter, ozone, nitrogen dioxide, sulpher dioxide, carbon monoxide, carbon dioxide.

- Contribute to the development & exacerbation of asthma & other respiratory diseases.
- Affect the respiratory mucosa chemically & immunologically.
- Enhance response to aeroallergens.
- Diesel exhaust particles increase the allergenicity of pollens.
- Epigenetic effects.

GLOBAL WARMING:

- More plants and pollens
- More mold & spores
- More stinging insects
- More ozone
- More stinging insects
- More outdoor activities

C. The Underlying Mechanism

In addition to allergies and asthma, modernization has been associated with dramatic rise in the prevalence of diabetes, rheumatic diseases, inflammatory bowel disorders, malignancies, and multiple sclerosis. All share a common underlying pathogenetic mechanism, namely immune dysregulation.

The impact of increasing exposures to various allergens and multiplicity of contributory factors seems to be enhanced by "cleanliness" and the development of the "Hygiene Hypothesis". There is a strong evidence that the Western life style with reduction in infections and parasitic diseases has favored the development of clinical allergies and asthma

- Imbalance in T-helper cell development towards Th2 > Th1.
- Increase in IL4 and IL13 that enhance IgE production.
- Increase in IL5 that enhance the development and activation of eosinophils.

"THE PROTECTIVE FARM EFFECT"

- Children who grew up in traditional farm environments developed less allergies and asthma; being attributed to early life contact with farm animals and microbes.
- Studies in Amish and Hutterite school children living on farms in the U.S. have further demonstrated that this protection is mediated through innate immune pathways.
- Although very similar with respect to ancestry genetics, Amish and Hutterites who follow farming practices were exposed to much higher levels of house dust endotoxins.

SUMMARY

Modernization has been associated with increased development of allergies and asthma. The driving factors are increased allergen exposures and multiple contributory factors. The main underlying mechanism is enhancing the T helper cell development towards Th2 > Th1.

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International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

IMMUNOTHERAPY IN ALLERGIC ASTHMA: WHERE DO WE STAND?

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Respiratory allergy is nowadays considered a single condition which affects both upper and lower airways, integrated in the "one airway" concept. The association of allergic rhinitis (AR) and asthma has been extensively established, and the common mechanistic pathways leading to inflammation also share multiple characteristics. Also, it is well-known that rhinitis frequently precedes the onset of asthma, allowing a window of opportunity for intervention [1]. Initially, allergen immunotherapy (AIT) was empirically developed to treat AR in 1911, mirroring the emergence of the first vaccines in infectious diseases. Nowadays, we understand that one of the inherent potential benefits of AIT is the simultaneous treatment of all clinical expressions of respiratory allergy, that is, from rhinoconjunctivitis to asthma [2]. This is a major advantage, compared to some of the other pharmacological therapies which selectively treat one of the target organs. Also, data indicate that AIT has a carry-over effect beyond the period during which it is administered, somehow reverting the clinical expression of the disease. Furthermore, it may also be able to exert a preventive effect in two modes: decreasing the risk of developing asthma in patients who only suffer AR and diminishing the tendency of the allergic patients to become sensitized to further allergens [3]. This review aims to provide a critical overview of the current knowledge on the effectiveness of AIT and its potential role in secondary prevention of respiratory allergy progression.

Key words: *respiratory allergy, allergic rhinitis, asthma, subcutaneous allergen immunotherapy*.

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UDC 615.37:616.248-056.3

Does immunotherapy treat asthma?

Subcutaneous allergen immunotherapy (SCIT) has extensively been evaluated in AR, since this has been the primary indication of AIT. Data provided by different meta-analysis of published trials have shown that it is an effective treatment, decreasing both symptom and medication scores [4–5]. In the last decades, sublingual immunotherapy (SLIT) has become a widely used form of AIT. Again, meta-analysis of studies have assessed its efficacy, indicating a reduction in symptoms and the need of medication [6–8]. Nevertheless, and despite meta-analyses are nowadays considered to hold the highest degree of evidence, critical appraisals have been published and should also be carefully considered [9–10].

But initial evidence on the effect of AIT in asthma came from small studies, with the primary objective to assess the efficacy for AR; therefore, they were underpowered to correctly evaluate the effect on asthma [11]. Nevertheless, results were good enough for further research, and trials in which the primary objective was to assess AIT impact on asthma followed. Assessment of AIT in asthma has only been adequately reported in studies designed to address this aim. In trials were the primary indication of AIT was rhinitis, it is difficult to infer the effect on asthma symptoms, either because they have not been examined, because patients with asthma symptoms were excluded, or because the low number of patients with asthma or the mildness of the disease renders underpowered results to draw conclusions [12–13]. Therefore, the effect of AIT on asthma has to be evaluated from specifically designed trials. A summary of the most relevant meta-analysis on the efficacy of AIT in asthma is shown in Table 1.

A recent Cochrane review on SLIT for asthma was unable to reach conclusions on the efficacy, due to the lack of data for important outcomes such as exacerbations and quality of life and use of different non-validated symptom and medication scores according to the authors [18]. On the contrary, other systematic reviews have been able to show a beneficial effect on symptoms and medication scores [7–8, 15]. These discrepancies may have been due to the different design of the studies and the choice of outcomes. As an example, in the mentioned meta-analysis on SLIT for asthma [18], the selected primary outcome for efficacy was exacerbation requiring emergency department visit or hospitalization; but this is an unlikely event to happen in well-conducted trials where patients are monitored and provided rescue medications precisely to avoid severe adverse events.

Data from recent large studies on SLIT have provided promising and more robust results both for rhinitis and

 ISSN 1562-3629

Table 1

Meta-analysis & systematic reviews on AIT for asthma

AIT	*Study*	*Patients*	*Population*	*Allergen/s*	*Symptom scores SMD (95%CI)*	*Medication scores SMD (95%CI)*
SLIT	Olaguibel 2005 [14]	193	Pediatric	Multiple	-1.42 (-2.51, - 0.34)*	Not reported
	Calamita 2006 [15]	1706	Pediatric & adult	Multiple	-0.38 (-0.79, 0.03)	-0.91 (-1.94, 0.12)
	Penagos 2008 [16]	441	Pediatric	Multiple	-1.14 (-2.10,- 0.18)*	-1.63 (-2.83, - 0.44)*
	Compalati 2009 [17]	476	Pediatric & adult	HDM	-0.95 (-1.74, - 0.15)*	-1.48 (-2.70, - 0.26)*
	Normansell 2015[18]	5077	Pediatric & adult	Multiple	Not reported**	Not reported**
	Liao 2015 [19]	454	Pediatric	HDM	-1.20 (-2.07, - 0.33)*	-0.52 (-1.75, 0.71)
SCIT	Abramson 2010 [20]	3459	Pediatric & adult	Multiple	-0.59 (-0.83, - 0.35)*	-0.53 (-0.80, - 0.27)*

*Statistically significant ($p<0.05$). **Authors felt unable to perform meta-analysis due to high variability of reporting and use of non-validated scores.

asthma. Regarding pollen AIT, different grass and ragweed tablet formulations have demonstrated efficacy in rhinoconjunctivitis [1–27], some studies reporting absence of asthma worsening during treatment [28–29], or even an improvement of asthma despite the study was not adequately powered for this assessment [30].

House-dust mite (HDM) tablets are also under development. A trial by Bergmann et al. in a group of 509 participants demonstrated a significant improvement in rhinitis symptoms during one year treatment with HDM tablets (300 and 500 IR *vs.* placebo), continuing in a one year follow-up [31]. The study by Mosbech et al. on the effect of a HDM-tablet (1.3 or 6 SQ *vs.* placebo) on allergic rhinitis and asthma included 604 patients. In the rhinitis patients, there was a 28.8% decrease in the total combined rhinitis score when comparing the 6 SQ-HDM treated group and placebo ($p<0.0357$), and an improvement in quality of life [32]. In the asthma study, a small reduction (-81 mcg) in the daily dose of inhaled corticosteroids necessary to control asthma was shown [33], which resulted larger (327 mcg) in a more severe sub-group of patients [34]. Further studies have confirmed the efficacy of this HDM-tablet in asthma, decreasing the probability of moderate and severe asthma exacerbations [35–36].

These pivotal studies have enabled asthma guidelines such as the Global Initiative for Asthma (GINA) 2017 to include this type of SLIT as a form of treatment for asthma [37]. A trial by Wang et al., enrolling 484 patients, was unable to prove a better outcome for the active group on HDM drop AIT, on the primary efficacy criterion which was well-controlled asthma during a period of inhaled steroid reduction [38]. But also in this study, a post-hoc analysis showed significant efficacy in the more severe subgroup (patients with moderate, but not mild, persistent asthma).

Safety outcomes of allergen immunotherapy

One of the major drawbacks of AIT is the potential induction of severe adverse events, specially asthma exacerbation or anaphylaxis.

Data from meta-analysis and large trials show that, taken together, SLIT shows a better safety profile than SCIT. In SCIT induction phases, the rate of systemic reactions is approximately 0.1% to 0.2% of injections and 2% to 5% of patients [39], while for SLIT, according to a review, the rate was 0.056% of doses [40]. Uncontrolled asthma has been identified as an independent risk factor for serious adverse effects [41–42]; therefore, it is of major importance to assess asthma status initially upon prescription of AIT, and thereafter, before administration, as recommended by national and international guidelines [39].

Final considerations

Allergic diseases are not confined to one organ; they are systemic conditions which should be managed as such. In the case of respiratory allergy it is well-known that rhinitis and asthma often coexist.

On the whole, current published data indicate that both SCIT and SLIT are effective for AR and asthma. Substantial evidence indicates a preventive effect in the progression from AR to asthma.

But we must be aware that these conclusions cannot be generalized: not all patients are the same and not all products are equivalent. In this era of precision medicine there is a need to phenotype the most adequate patients who will benefit from AIT, taking into account among other characteristics, severity and specific sensitization profiles [43–44]. Also AIT products vary in quality, potency and molecular allergen content [45], so the clinical effect is not homogeneous.

In the context of the "one airway" concept, when evaluating the effect of AIT, it is appropriate to consider results affecting both the upper and the lower airways. Indeed, in trials addressing AR, adverse effects, including asthma exacerbations are usually reported. But most rhinitis studies specifically exclude patients with asthma, unless it is mild. Therefore, any therapeutic effect of AIT on asthma in these patients is intrinsically limited.

On the other hand, some studies are designed to assess asthma as the primary indication. Nevertheless, many asthma trials also suffer of several limitations. One is disease severity of patients enrolled in trials, which usually is mild and requiring low dose of inhaled corticosteroids and rescue bronchodilators, allowing for little improvement. As has been mentioned, probably slightly more severe patients may benefit more from AIT [34, 38], just like more severe rhinitis.

Another limitation is that there is no consensus on which are the most adequate study designs and outcomes to

assess AIT efficacy in asthma. There is a lack of validated symptom and medication scores, or a unanimous definition of asthma exacerbations.

The consequence is a great variability of results, difficult to compare or to pool for meta-analyses; this should prompt scientific societies to develop guidelines on the most appropriate designs to further evaluate AIT in asthma.

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Meeting Calendar

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April 20–23, 2018 Barcelona, Spain

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International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

MOLECULAR DIAGNOSIS IN RESPIRATORY AND FOOD ALLERGY

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The knowledge on molecular allergy diagnosis is continuously evolving. It is now time for the clinician to integrate this knowledge and use it when needed to improve the accuracy of diagnosis and thus provide more precise therapeutic and avoidance measures. This review does not intend to comprehensively analyze all the available allergen molecules, but to provide some practical clues on use and interpretation of molecular allergy diagnosis. The potential role of component resolved diagnosis in circumstances such as the indication of allergen immunotherapy, pollen polysensitization, food allergy, latex allergy or anaphylaxis, is assessed. Interpreting the information provided by molecular allergy diagnosis needs a structured approach. It is necessary to evaluate single positivities and negativities, but also to appraise "the big picture" with perspective.

Key words: *allergen immunotherapy, pollen polysensitization, food allergy, latex allergy.*

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UDC 636.2.034: 616-056.3: 12.017

Indication of specific immunotherapy

The first premise for the prescription of immunotherapy based on CRD is the assessment of IgE positivity to genuine versus cross-reactive allergens (Fig. 1 and 2).

INHALANT OLIGO/MONOSENSITIZATION. Precise identification of relevant sensitizers in the case of pollen-allergic patient's mono or oligosensitized to pollens with no overlapping pollen season can be achieved by conventional diagnosis with complete pollen extracts. In most cases patients are sensitized to major pollen allergens (e.g. Ole e 1, Bet v1, Phl p1/Phl p5), but this may not be the case in areas with high pollen loads, for example to olive pollen in the south of Spain, where Ole e 7 and Ole e 9, currently considered as minor allergens, can be the major sensitizers [1]. When prescribing specific immunotherapy in areas with high frequency of sensitization to "minor allergens", molecular diagnosis may be of special interest, since commercial extracts for immunotherapy are well standardized only for major allergens. Thus, patients with sensitization to minor allergens alone may likely not receive sufficient amounts of allergen to achieve a successful outcome from allergen immunotherapy [AIT], or even worse, will experience adverse reactions when the concentrations of this minor allergens present in the extract are high [2].

Another scenario in which molecular allergy would help to decide the correct indication of AIT would be dog dander allergy. Unlike cat allergy, almost all attributable to sensitization to its major allergen Fel d 1, the sensitization profile in case of dog allergy is more heterogeneous [3]. In Spanish populations, Can f 5 is a major allergen, with reported sensitizations of 70% of dog allergic patients [4]. In our series, Can f 1, 2 and 3 are minor allergens, while Can f 5 is responsible for up to 67% of sensitizations and, importantly 37% of our patients are not sensitized either to Can f 1, 2 or 3 but only to Can f 5 [5]. There is a high variability between commercial dog extracts regarding their allergen contents [6], and Can f 5 is poorly represented. It would not seem appropriate to indicate specific immunotherapy to dog extract in patients monosensitized to Can f 5 until this (and possibly other) major allergen content is guaranteed in the therapeutic extract.

POLLEN POLYSENSITIZATION. Unfortunatelyб mono/oligosensitized patients are more and more scarce, at least in adults whose diagnostic complexity increases with polysensitization [7].

Although most studies on the relevance of CRD in complex pollen areas have been performed in the south of Europe, polysensitization to respiratory allergens is also seen in the north. This has been shown by data of the European Community Respiratory Health Survey (ECRHS) where 12.8% to 25.3% of patients were polysensitized [8]. This fact has important implications when considering the prescription of immunotherapy. In recent large clinical trials, single-allergen immunotherapy with grass pollen extract has proved to be as safe and effective in polysensitized as in monosensitized patients [9–10], provided that the allergen extract administered matches the patient's most relevant sensitization.

CRD provides the information on patient specific allergen sensitization to drive the selection of the immunotherapy extract [11], conceptually "component-resolved treatment". AIT would be appropriately prescribed if sensitization to the species-specific allergens is confirmed, while in case of selective recognition of cross-reactive allergens, like profilins or CCD, the indication of AIT is arguable. Cross-

 ISSN 1562-3629

reactive allergens seem to have limited clinical relevance and their content in AIT extracts is usually not quantified. Also, if all components are negative, sensitization to the allergen source cannot be ruled out, but AIT extracts would be unlikely to contain the sensitizing molecule.

Proving the importance of a CRD-driven immunotherapy prescription, three prospective studies, including adult and pediatric population, have recently shown that the incorporation of CRD results alters initial AIT prescription in approximately half of the patients [12–14]. However, there is still a gap between the current possibility of using a predefined AIT preparation and the complexity of sensitization at the population level, since the patients sIgE profiles are highly heterogeneous depending on the geographical area and the allergen source [15–16]. Also, there is a need to adequately evaluate in prospective studies if CRD- guided patient selection results in improved efficacy of immunotherapy.

Since the first proposal of Valenta [17], few articles have been published on how to use CRD results for the optimal selection of immunotherapy. Very recently Douladiris et al. have proposed a comprehensive and practical algorithm regarding component-resolved diagnostic work-up for pollen AIT candidates in southern Europe [18].

Food allergy

Risk assessment. Since the first the study on CRD in apple allergy across Europe [19], where it was demonstrated that sensitization to apple nsLTP (Mal d 3) was associated with a 7 fold risk of anaphylaxis compared to sensitization apple Bet v 1 homologue (Mal d 1), nsLTP have been considered markers of severe allergic reactions. However, studies on patterns of nsLTP sensitization in Mediterranean patients have shown that the clinical expression is variable, ranging from asymptomatic sensitization to severe anaphylaxis [20–21], possibly modulated by pollen allergen co-sensitization and the presence of cofactors [22] A predictive pattern of clinical expression in nsLTP-sensitized patients has not yet been elucidated.

Bet v 1 homologues are considered markers of mild allergic reactions to fruits and vegetables due to cross-reactivity with birch pollen. However, although not frequent, some anaphylactic reactions to apple in patients sensitized to PR10-proteins have been reported [23]. In the particular case of soya allergy, Gly m 4 (the Bet v 1 related allergen in soya) has been related to severe, generalized symptoms [24].

Storage proteins from nuts and soya have been associated with higher risk of severe allergic reactions [25]. In the case of peanut, Ara h 2 seems to be the best predictor of peanut allergy, reducing the need for peanut challenges by at least 50% [26]. Altogether, Ara h 1, Ara h 2 and Ara h 3 have been associated with severe symptoms, although anaphylactic reactions have been described in patients negative for these allergens[27].

Sensitization to Cor a 9 and Cor a 14 have been reported to be highly specific for hazelnut allergic patients with objective symptoms in DBPCFCs and proposed as markers for a more severe hazelnut allergic phenotype[28]. Similarly, in patients with soybean allergy, Gly m 5 and Gly m 6 have been proposed as potential markers for severe allergic reactions [29].

Altogether, CRD may be a useful tool for stratifying patient's risk for severe reactions but it is important to bear in mind that the risk to develop anaphylaxis depends not only on the allergens an individual patients is sensitized to, but also the degree of sensitization, the quality of binding allergens, the immunoglobulin affinity, the route of application and the presence of cofactors [30]. Figure 1 depicts those allergens that have been associated to higher versus lower risk of anaphylaxis.

Polysensitization to inhalant and food allergens

One of the biggest challenges for the allergist is to confront the patient with positive SPT to several pollen and food allergens. In this scenario CRD may be of major usefulness, improving the resolution of conventional diagnosis by adding information on the genuine primary sensitizers to distinguish them from sensitization due to cross-reactivity [31] (Figures 2 & 3). With regard to poly-pollen sensitization, this information may be relevant for prescribing AIT as discussed before.

Polysensitization to animal dander (cat, dog and horse) can in part be explained by cross- reactive lipocalins and albumins (3). Serum albumin is also implicated in cross-reactivity in the so-called cat-pork syndrome, where patients developing a cat serum albumin IgE response react upon pork meat ingestion [32].

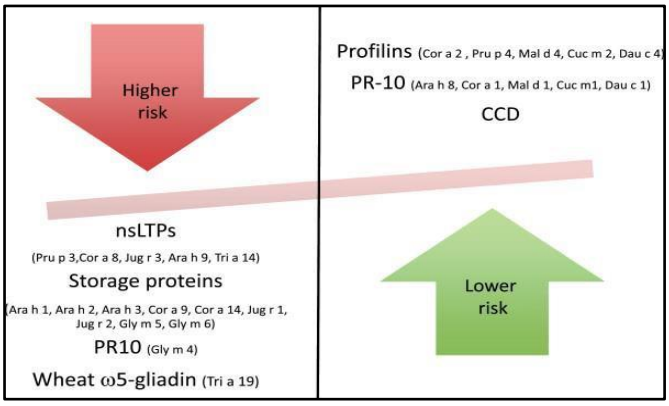

Fig. 1. Allergens associated to higher versus lower risk of anaphylaxis. *CCD: Cross-reactive carbohydrate determinants; nsLTP: Non-specific lipid transfer proteins.*

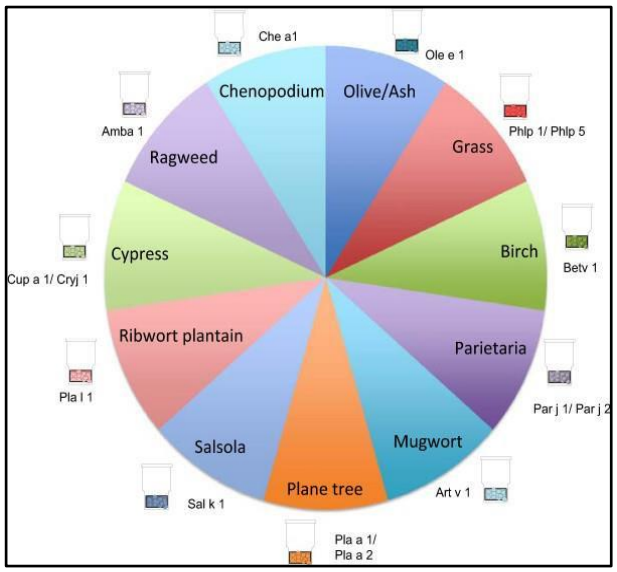

Fig. 2. Pollen species-specific allergens.

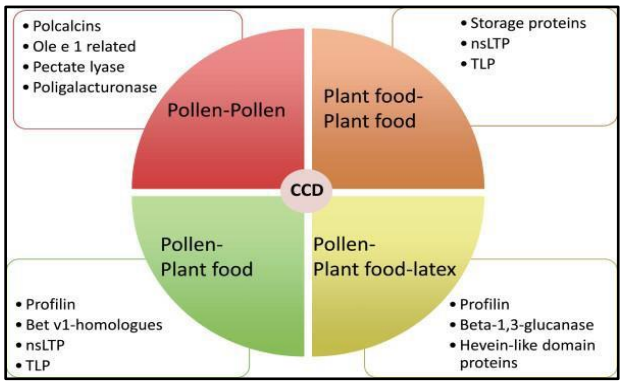

Fig. 2. Cross-reactive allergens. CCD: Cross-reactive carbohydrate determinants; nsLTP: Non-specific lipid transfer proteins; TLP: thaumatin-like proteins.

Allergic reactions to fruits and vegetables can result from a primary sensitization to food or to inhalant allergens. Usually, cross reactivity is attributable to labile allergens (e.g., PR-10 and profilins) and associated with mild oral reactions [33], while heat and proteolysis-resistant allergens that primary sensitize through the oral route, are associated with systemic reactions in addition to local reactions (e.g. seed storage proteins and nsLTP) [34]. Sensitization to CCD in food or venoms does not have remarkable clinical relevance, and the primary sensitization may derive from either pollen or venoms [35]. Since purified native allergens may express carbohydrates (while recombinants do not), determination of sIgE to MUXF3 (a type of CCD) should be performed to rule out irrelevant sensitization to CCD in case of positive sIgE to purified native glycosilated allergens without clinical symptoms [36].

Anaphylaxis

COFACTOR-ENHANCED FOOD-DEPENDENT ANAPHYLAXIS. Epidemiological data show that cofactors are relevant in up to 39% of all food-dependent anaphylactic reactions in adults [30]. Wheat dependent exercise-induced anaphylaxis (WDEIA) is the best characterized of these syndromes, classically related to omega-5-gliadin sensitization. Recently it has been reported that, at least in the Mediterranean population nsLTP accounts for the majority of cofactor-enhanced food allergy (CEFA) [37–38), mainly related with vegetables, nuts and cereals. Even in cases of WDEIA reactions, positivity to nsLTP in the absence of omega-5-gliadin sensitization has been reported [39]. Therefore, at least in southern Europe, patients with a history consistent with CEFA anaphylactic reactions should be tested for sIgE to nsLTP (mainly Pru p 3, but also to Tri a 14) and to omega-5-gliadin. Other underlying sensitizations may be relevant in some populations.

RED MEAT DELAYED ANAPHYLAXIS. When evaluating a patient with a history of delayed onset anaphylaxis 3–6 h after ingestion of mammalian food products (e.g., beef and pork), sIgE against galactose-α-1,3-galactose (α-gal) should be performed [40]. Before the identification of the allergen responsible for this syndrome, because of the delay of symptoms after ingestion of meat products, the frequent negative SPT responses and the good tolerance to other meats like turkey, these types of anaphylaxis have been wrongly classified as idiopathic [41]. It has been suggested that tick bites are the cause of IgE antibody responses to α-gal and it is recommended to reassess sIgE levels every 8 to 12 months as they tend to decrease over time, and some patients have been able to tolerate mammalian meat again after avoiding additional tick bites for 1 to 2 years [42].

IDIOPATHIC ANAPHYLAXIS. Although idiopathic anaphylaxis involves a small proportion of patients with anaphylaxis, the clinical implications are highly significant. The inability to identify a cause prevents from usual anaphylaxis interventions such as avoidance measures, specific education and modification of risk.

To date, only one study has addressed the question whether the ISAC allergy array would add diagnostic value in patients with idiopathic anaphylaxis. Heaps et al [43] performed an ISAC-103 test (Thermo Fischer Scientific, Uppsala, Sweden) to 110 patients with a diagnosis of idiopathic anaphylaxis in UK and found new allergenic sensitizations in half of the patients studied, which in 20% of the cases were identified as the cause of the anaphylaxis with a high likelihood (although it was only reassessed in 50% of those patients). Omega-5-gliadin and shrimp allergens accounted for 45% of the previously unrecognized sensitizations. Other newly identified allergens related to the anaphylaxis were seed storage proteins, nsLTP and latex allergens.

We must bear in mind that some molecules are poorly represented in allergen extracts, and therefore the sensitivity of conventional diagnostic tests (SPT, sIgE) will not allow a diagnosis.

Therefore, the performance of a multiplex CRD and sIgE to α-gal would be very helpful in the assessment of idiopathic anaphylaxis. If positive, it may orientate on the triggering allergen; if negative, a non-IgE mediated mechanism underlying the anaphylaxis may be more likely.

ISSN 1562-3629

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Meeting Calendar

♦

May 26–30, 2018 Munich, Germany

EUROPEAN ACADEMY OF ALLERGOLOGY AND CLINICAL IMMUNOLOGY(EAACI) CONGRESS

♦

June 15–19, 2018 Tbilisi, Georgia

VI EUROPEAN CONGRESS ON ASTHMA, COPD AND RESPIRATORY ALLERGY

♦

CONGRESS SECRETARIAT
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ISSN 1562-3629

International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

HUMAN EXPOSURE TO FUNGI AND ITS RELEVANCE TO HEALTH

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Fungi are ubiquitous microorganisms that are present in outdoor and indoor environments, with more than 100,000 species in the world, fungi can be found everywhere. When airborne, fungi take the form of spores, mycelia and hyphae fragments. Such bio particulates, when inhaled, are believed to contribute to adverse health effects in individuals who are predisposed. Individual fungal species contain 40 or more allergens for human. However the sensitivity is specific, but cross-reactivity is a common feature of fungal allergy. Respiratory diseases by inhalation of fungi spores can be atopic asthma, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis and allergic fungal sinusitis. Mechanisms for the pathogenesis of human disease caused by fungi are discussed.

Key words: *antigenicity, immune system, heterotrophic organisms, fungi spores, allergens.*

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UDC 616-056.3-053

Fungi are ubiquitous microorganisms that are present in outdoor and indoor environments, with more than 100,000 species in the world, it is no wonder fungi can be found everywhere. They are heterotrophic organisms which do not use light for photosynthetic assimilation process. They lack chlorophyll. Many species live and stay invisible during mostly of their biologic cycle.

They are microscopic organisms that produce enzymes to digest organic matter and spores to reproduce and in nature, fungi play a key role in the natural biologic recycling by digesting and degrading organic substrates to chemically more simple components. Only their spore forming tissue systems become visible as moldy of mildewed surface (toad stools or mushrooms).

Fungi are ubiquitous microorganisms that can be found in all parts of the world. When airborne, fungi take the form of spores, mycelia and hyphae fragments. Such bio particulates, when inhaled, are believed to contribute to adverse health effects in individuals who are predisposed.

Filamentous organisms that absorb food from the environment after external digestion and their cells walls are made of a variety of materials that carry some antigenicity. Glucans in the cell wall are endotoxin-like and irritating and stimulate the immune system.

They live outdoor and indoor and requirements for fungus growth is simple like moderate or high relative humidity (>60%), temperature between 4–38°C, and organic matter.

Indoor growing problems arise when fungus starts digesting organic materials we do not want them to, like our homes. They need moisture to begin growing and digesting whatever they are growing on. Also, some can grow on wood, ceiling tiles, wallpaper, paints, carpet, sheet rock, and insulation. When excess moisture or water builds up with high humidity, or flooding, conditions are often ideal for fungus. Realistically, there is no way to rid all fungi and fungi spores from our home; the way to control fungi growth is to control moisture.

Fungus is a naturally occurring organism that produces seed-like spores that are small enough to travel through the air. We are exposed to fungus daily in the air we breathe, or through ingestion, and/or skin contact. The level of airborne fungus increases when moisture problems arise in buildings creating fungus growth on building materials.

Outdoor variations in the air spore are due to diurnal rhythms of spore release (ecology), which is different on various fungi, weather and special related factors.

Indoor fungi measures can be evaluated by air sampling or source sampling by total counts or colonies (CFU). In table 1 the more prevalent fungi either outdoor as indoor is showed [1]. Aerobiology can be a valuable tool for estimating bioaerosol exposure to fungi and immunochemical and molecular methods are useful for detecting specific fungi from air sample. These techniques as monoclonal antibodies and DNA probes are available for fungi with known health effects.

Atmosphere contains a large variation of fungi spores, in morphology and in ecology and building and houses also contains a large variation of fungi spores. The concentration of environmental allergens depends on many variables, including climate, vegetation and air quality. Outdoor allergens are predominantly plant pollens and fungal spore.

Indoor allergens include proteins from dust mite, cockroach, animal dander and fungal spores and concentration are affected by humidity, ventilation and presence of pets, carpets or houseplants. Individual fungal species contain 40 or more allergens for human. However the sensitivity is specific, but cross-reactivity is a common feature of fungal allergy. Deuteromycetes is most allergenic fungal genera that induce IgE sensitization.

Airborne spore's fungi have minimal geographic variations compared with pollens and there are no seasonal pat-

Table 1

Outdoor fungus and Indoor adapted from D´Amato G., Spieksma F.

Outdoor			*Indoor*
Cladosporium	40–80	600,000	*Cladosporium*
Basidiospores	5–30	25,000	*Penicillium*
Ascospores	5–20	15,000	*Alternaria*
Yeasts, *Sporobolomyces*	2–20	15,000	*Aspergillus*
Botrytis	2–20	12,000	*Botrytis*
Aspergillus, Penicillium	2–20	10,000	*Wallermia*
Alternaria	1–10	7,500	Yeasts
Didymella	1–10	7,500	*Mucor*
Fusarium	1–10	7,500	*Epicoccum*
Ustilago	1–10	7,500	Basidiospores

terns among fungi compared with pollens. We found the prevalence of indoor fungus [2] in schools with >100 CFU/m^3 from *Cladosporium, Alternaria, Aspergillus, Agaricus, Coprinus, Ustilago, Stemphylium* and *Botrytis*. However, in houses of people with low income, there are higher prevalence of spores like 320,000 CFU/g for the bedrooms and 820,000 CFU/g for the living rooms, with mean concentration 14,400 CFU/g (bed and living room).

The fungi identified and associated with asthma (*Alternaria, Aspergillus, Penicillium, Cladosporium*, Mucor, Rhizopus, and *Ulocladium*) maximum values were 136,000 CFU/g for the bedrooms and 32,000 CFU/g for the living rooms [3].

In healthy individuals, most fungi are not pathogenic, usually provokes superficial infections of the feet, groin, nails and skin are the most common "infection".

Nevertheless, only a limited number of fungi like *Blastomyces, Coccidiodes, Cryptococcus*, and *Histoplasma* can cause a severe illness in healthy individuals.

The extent to which an individual may be affected depends on susceptibility to disease, the organism and duration and severity of the exposure.

We can say that we have different mechanisms for the pathogenesis of human disease caused by fungi:

- Irritation by fungi components known to elicit a response in humans such as VOC (MVOC): headaches, attention deficit, dizziness, and mucous membrane irritation and β-glucans that can cause allergic reactions in humans: mild and moderate rhinitis or conjunctivitis, chronic sinusitis and sometime asthma.
- Infection, in those that are at risk for systemic fungal infections (*Aspergillus, Cryptococcus neoformans* and *Candida albicans*, like severely immunocompromised individuals, undergoing chemotherapy, have had organ or bone marrow transplants and with AIDS. However, infection by dermatophytic fungi is reasonably common, causing superficial infection of the skin, nails, hair and mucous membranes.

Fungi can produce allergens, irritants and in some cases toxins. Common reactions to mould are: cough, congestion, runny nose, burning eyes, headaches, sneezing and sore throat and children, pregnant women, the elderly and people with weak immune systems may be more sensitive to mould. It is estimated that ~10% of the general population have IgE antibodies to common inhalants fungi and ~ 50% of these are predicted to have, at the same time, allergic symptoms related to exposition to fungi.

Sensitization to fungi, particularity *Alternaria*, has been linked to the presence, persistence, and severity of asthma. Infants at risk for asthma, in the first year of life, defined by a maternal history of asthma, that are exposed to high concentration of fungi, have high risk to develop persistent cough and wheezing.

Respiratory diseases by inhalation of fungi spores can be atopic asthma, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis and allergic fungal sinusitis.

Atopic patients commonly have IgE antibodies to fungi as part of polysensitization, and allergic responses to inhaled fungi antigens are a recognized factor in upper and lower airway disease. However, exposure to airborne fungi is not recognized as a contributing factor in atopic dermatitis, as well as urticaria, angioedema or anaphylaxis.

Most of the identified fungal allergens are proteins associated with essential basic fungal metabolism such as protein synthesis and or glycolysis like *Alternaria alternata* – Alt a 1, *Cladosporium herbarum* Cla h 6 and *Aspergilus fumigatus* Asp f 1.

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 ISSN 1562-3629

International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

WHAT'S NEW IN ALLERGY, ASTHMA AND COPD?

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In this report, recent advances in asthma, COPD, drug hypersensitivity, food allergy, immunodeficience, angio-edema and urticaria are discussed.

Key words: *asthma, COPD, children, inhaled glucocorticoids, respiratory symptoms, hypersensitivity reactions.*

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UDC 616.233-007:171-08-039

ASTHMA AND COPD

SEVERE EOSINOPHILIC ASTHMA (JANUARY 2018)

Benralizumab, a monoclonal antibody against interleukin-5 receptor alpha has been approved by the FDA as add-on therapy in patients aged 12 years and older with severe eosinophilic asthma [1]. Benralizumab allowed tapering of oral glucocorticoids and reduced exacerbations in patients with peripheral blood eosinophils ≥150/microL compared with placebo.

LONG-ACTING BETA AGONIST-GLUCOCORTICOID COMBINATION INHALERS: FDA BOXED WARNING REMOVED (JANUARY 2018)

Recently the FDA, based on a review of four large clinical safety trials concluded that long-acting beta agonist (LABA)-inhaled glucocorticoid combination inhalers do not significantly increase the risk of serious asthma-related side effects compared with inhaled glucocorticoids [2] but continues to warn that the use of LABA alone is contraindicated in the treatment of asthma.

TIOTROPIUM FOR SEVERE SYMPTOMATIC ASTHMA IN CHILDREN (DECEMBER 2017)

In a 12-week randomized trial in 400 children (aged 6 to 11 years) with severe symptomatic asthma, once-daily tiotropium 5 mcg, improved lung function compared with placebo and was well tolerated as add-on therapy to inhaled glucocorticoids and other maintenance therapies [3].

THE GLOBAL INITIATIVE FOR CHRONIC OBSTRUCTIVE LUNG DISEASE (GOLD) RELEASED A NEW "2017 REPORT".

The new report contains modified recommendations for the diagnosis, management, and prevention of COPD. Definition of COPD has been changed: "COPD is a common, preventable, and treatable disease that is characterized by persistent respiratory symptoms and airflow limitations that are due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases." The description of the pathophysiology of COPD is more elaborate and more detailed and the terms "emphysema" and "chronic bronchitis" have been removed.

The new guidelines include symptom severity and exacerbation risk to classify COPD.

COPD medications are extensively discussed in the 2017 Report which recommends escalation strategies [4].

EXHALED NITRIC OXIDE ANALYSIS AND CHRONIC COUGH (OCTOBER 2017)

In a systematic review of over 2000 patients, the fraction of exhaled nitric oxide FENO was more useful to confirm and include cough-variant asthma and non-asthmatic eosinophilic bronchitis than to exclude them [5].

GLOBAL ASTHMA MORTALITY (OCTOBER 2017)

Asthma is the thirty second cause of death globally, with different mortality rates in different countries. World Health Organization data have shown that asthma mortality didn't change from 2006 to 2012 [6]. Lower mortality rates are related to better practice and implementation of asthma management strategies.

DRUG HYPERSENSITIVITY

HYPERSENSITIVITY REACTIONS WITH ROLAPITANT (JANUARY 2018)

Post marketing reports have shown that Rolapitant, a neurokinin-1 receptor antagonist, used to prevent nausea and vomiting associated with cancer chemotherapy, can cause anaphylactic shock and serious hypersensitivity reactions in patients receiving intravenous rolapitant emulsion, which is in soybean oil. Patients with known allergies to soybeans, legumes, or other related allergens should be monitored closely [7].

CYCLOSPORINE FOR STEVENS-JOHNSON SYNDROME/TOXIC EPIDERMAL NECROLYSIS (NOVEMBER 2017)

Beyond supportive care, there are no established therapies for Stevens-Johnson syndrome/toxic epidermal necrolysis. Recently cyclosporine has been shown to slow the progres-

sion of the disease. The authors suggest a beneficial effect of cyclosporine in addition to the classic supportive care [8].

INFLUENZA VACCINATION IN INDIVIDUALS WITH EGG ALLERGY (DECEMBER 2017)

The 2017 update of guidelines from the American Academy of Allergy, Asthma, and Immunology (AAAAI)/American College of Allergy, Asthma, and Immunology (ACAAI) Joint Task Force on Practice Parameters no longer recommends inquiring about egg allergy before influenza vaccine administration [9]. Individuals with egg allergy of any severity should undergo yearly influenza vaccination administered in the usual manner according to standard indications and contraindications, without special precautions.

IDENTIFICATION OF CHILDREN WITH LOW-RISK PAST PENICILLIN REACTIONS (AUGUST 2017)

The authors consider both hives and angioedema as high-risk features and suggest that children with past reactions involving either of these symptoms be referred to an allergy specialist to determine if penicillin can be safely used [10].

FOOD ALLERGY

MULTIFOOD ORAL IMMUNOTHERAPY (OIT) PLUS ANTI-IGE (DECEMBER 2017)

The results of a study involving 48 children aged 4 to 15 years with two to five food allergies, suggest that the addition of anti-IgE to multifood OIT improves both efficacy (by enabling more rapid desensitization) and safety [11].

PEANUT EPICUTANEOUS IMMUNOTHERAPY (DECEMBER 2017)

Epicutaneous immunotherapy (EPIT) is an investigational approach for delivering immunotherapy that solubilizes the allergen by perspiration and delivers it into the skin. In a trial comparing EPIT (at different doses) with placebo, all groups demonstrated some degree of treatment success [12].The greatest effect was seen in children aged 6 to 11 years.

PROBIOTIC PLUS ORAL IMMUNOTHERAPY FOR PEANUT ALLERGY (AUGUST 2017)

In a follow-up study four years after completion of a randomized trial and cessation of treatment, patients treated with peanut OIT plus probiotic were more likely to have sustained unresponsiveness to peanut after eight weeks of avoidance compared with patients treated with placebo only [13].

PROBIOTICS INEFFECTIVE FOR THE PREVENTION OF EARLY CHILDHOOD ECZEMA (OCTOBER 2017)

Previous data suggested that probiotics used in late pregnancy/early infancy had some protective effect on the development of eczema in the first year of life. A recent randomized trial provides evidence of the lack of effectiveness of probiotics for eczema prevention [14].

NATIONAL ACADEMIES CONSENSUS REPORT ON FOOD ALLERGIES (AUGUST 2017)

The National Academies of Sciences, Engineering, and Medicine consensus report on food allergies highlights a number of critical issues related to food allergy [15].

- Judicious use of food allergy testing performed and interpreted in the context of the patient's clinical history.
- Prompt treatment of anaphylaxis with epinephrine.
- Primary prevention of peanut allergy through early dietary introduction.

IMMUNODEFICIENCY

CONSENSUS STATEMENT ON GRANULOMATOUS AND LYMPHOCYTIC INTERSTITIAL LUNG DISEASE (AUGUST 2017)

Granulomatous and lymphocytic interstitial lung disease (GLILD) is the most common cause of diffuse parenchymal lung disease in patients with common variable immunodeficiency. The British Lung Foundation and UK Primary Immunodeficiency Network published a consensus statement summarizing the experience of physicians caring for patients with GLILD [16].

URTICARIA AND ANGIOEDEMA

RECOMBINANT C1 INHIBITOR (RHC1INH) FOR PREVENTION OF HEREDITARY ANGIOEDEMA ATTACKS (OCTOBER 2017)

RhC1INH is used for treatment of acute episodes of hereditary angioedema. A multicenter randomized trial of 26 patients with high attack rates demonstrated that rhC1INH decreased the mean number of attacks per month, compared with placebo [17]. However its shorter half-life limits its use in prophylaxis.

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ISSN 1562-3629

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Meeting Calendar

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September 2–5, 2018 Amsterdam, The Netherlands

EUROPEAN CONGRESS ON IMMUNOLOGY (EFIS)

♦

September 15–19, 2018 Paris, France

EUROPEAN RESPIRATORY CONGRESS

♦

October 2–6, 2018 Tokyo, Japan

WORLD ASTHMA CONGRESS

♦

International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

AIR POLLUTION AND CLIMATIC CHANGES EFFECTS ON PATIENTS WITH BRONCHIAL ASTHMA

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Epidemiological studies shown that the prevalence of asthma has risen dramatically worldwide and evidence suggests that air pollution factors have an important role in the etiology of the disease. The study aimed to establish the correlation between the concentration of phadiatop, total IgE levels in the blood in patients with diagnostic bronchial asthma and the concentration of specific air pollutants in terms of annual calendar of flowering plants in West Georgia.In the study were involved 45 patients (among them 24 males and 21 females) of different ages, with diagnostic bronchial asthma (according to GINA recommendation)who for allegro-specific diagnostics applied to the S/R Institute of Allergology, Asthma and Clinical Immunology of Georgian Academy of Sciences (Tskaltubo, Georgia) from January to April, 2017. The study included the following stages of allegro-diagnostics:I step– allegro diagnostic using modern automated system – "Immuno CAP 100" (PHADIA, Switzerland) II step – Monitoring of aeropollutants concentration by using aeropolinometer Burkard Trap (Great Britain).The analysis of the laboratory results showed that the studied patients had high titers of total IgE, which amounted to an average of 273 (N 33–90), while the average concentration of phadiatop was 96 (N<70), respectively. In the patients with bronchial asthma of a specific positivity of specific IgE to the weeds (Wx2) – ambrosia, plantain, clasp/tarragon, atriplex –in 25 (55%) on average; tree dust (Tx9) – alder, lactariuspiperatus, nuts, oak, willow – 16 (35%); and cereals (Gx1) – festucapratensis, loliumtemulentum, timoti grass, poa – 8 (17%); Mx2 – Penicilliumnotatum, Cladosporiumherbarum, *Aspergillus fumigatus, Candida albicans, Alternaria alternate* – 11 (24%) was revealed, only in 6 (13%) patients we cannot established the allergy specific IgE. From January to April 2017, there were revealed a high concentration of aeropollutants, by high allergenization and widespread; especially high concentrations were found in alder, birch tree and common hazel, while from aeropollutants of low allergenization poplar, elm, willow and plane tree were distinguished. A concentration of different types of tree-dust and surrounded atmospheric aeropollutants was specified by using aeropolinometer Burkard Trap at a given period of time and consequently, the annual calendar for distribution of aeroallergens in West Georgia was developed over again. High degree correlation between the above-mentioned markers proves its clinical importance/value with respect to bronchial asthma.

Key words: *bronchial asthma, risk factors, air pollution, climate changes, Burkard Trap, specific IgE.*

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UDC 614.7:616.248-08.

Interest in air pollution and its effects on health and disease has been increasing steadily. A majority of physicians and scientists have been impressed that air pollution cannot help but aggravate diseases of the upper and lower respiratory tracts, although it has been questioned whether there is firm evidence that this is an established fact; some have called air pollution "a cause in search of a disease." The intent of this review is to describe concisely the current state of knowledge about the effects of air pollution on asthma, with the understanding that the primary etiologic factors in the asthmatic response are generally not characterized for the study populations in the reports to be reviewed. The major components of "air pollution" will be described with the understanding that they exist to varying degrees in different communities and atmospheric conditions, air pollutants do not occur as individual entities but in combination, frequently with synergistic effects, and there may be other pollutants or adjunctive factors equally important whose role is not yet appreciated.

Epidemiological studies shown that the prevalence of asthma has risen dramatically worldwide and evidence suggests that environmental factors have an important role in the etiology of the disease. Air pollution exposure is associated with increased asthma and allergy morbidity and is a suspected contributor to the increasing prevalence of allergic condition. Observation studies continue to strengthen association between air pollution and asthma. Although the fundamental causes of asthma are not completely understood, the strongest risk factors for developing asthma are inhaled asthma triggers.

ISSN 1562-3629

Airopolinologic study and monitoring in modern clinical medicine acquired particular importance, as far as it is known that among the etiologic factors of bronchial asthma the highest percentage comes on plant allergens-aeropollutants represented in the form of dust of plants and herbs (ambrosia, alnus, birch, maple, walnut, mallow, cotton and etc.) [6].

Climate and geographical conditions such as: air temperature, humidity and plant diversity represented in the region is of great importance for prevalence of allergic diseases.

Since airborne allergens and air pollutants are frequently increased contemporaneously in the atmosphere, an enhanced IgE-mediated response to aeroallergens and enhanced airway inflammation could account for the increasing frequency of respiratory allergy and asthma in atopic subjects in the last 5 decades. Pollen allergy is frequently used to study the relationship between air pollution and respiratory allergic diseases, such as rhinitis and bronchial asthma.

Epidemiologic studies have demonstrated that urbanization, high levels of vehicle emissions, and westernized lifestyle are correlated with an increased frequency of respiratory allergy prevalently in people who live in urban areas in comparison with people living in rural areas. Climatic factors (temperature, wind speed, humidity, thunderstorms, etc.) can affect both components (biological and chemical) of this interaction.

All these factors will maintain growing of allergization of the body [1–2, 7].Georgia is famous for its diverse flora and landscapes, the eco-geographic climate varies across the Eastern and Western Georgia, clinical and epidemiological studies have also proved that the Western Georgia is distinguished by the frequency and diversity of allergic diseases, especially the high rate of bronchial asthma and their acute and long-term process [3–6].

According to the above-mentioned, at this stage the study aimed to establish the correlation between the concentration of phadiatop, total IgE levels in the blood in patients with diagnostic bronchial asthma and the concentration of specific air pollutants in terms of annual calendar of flowering plants.

Materials and Methods

In the study were involved 45 patients (among them 24 males and 21 females) of different ages, with diagnostic bronchial asthma (according to GINA recommendation)who for allegro- specific diagnostics applied to the S/R Institute of Allergology, Asthma and Clinical Immunology of Georgian Academy of Sciences (Tskaltubo, Georgia) from January to April, 2017.

The study included the following stages of allegrodiagnostics:

I step: To detect allergenization degree, total serum IgE levels, specific IgE and concentration of Phadiatop, using modern automated system – "Immuno CAP 100" (Phadia, Switzerland), were estimated in the patients.

II step: Monitoring of aeropollutants concentration was conducted by using aeropolinometer Burkard Trap (Great Britain).

Results

The analysis of the laboratory results showed that the studied patients had high titers of total IgE, which amounted to an average of 273 (N 33–90), while the average concentration of phadiatopwas 96 (N<70), respectively.

In the patients with bronchial asthma of a specific positivity of specific IgE to the weeds (Wx2) – ambrosia, plantain, clasp/tarragon, atriplex –in 25 (55%) on average; tree dust (Tx9) – alder, lactariuspiperatus, nuts, oak, willow – 16 (35%); and cereals (Gx1) – festucapratensis, loliumtemulentum, timoti grass, poa – 8 (17%); Mx2 – Penicilliumnotatum, Cladosporiumherbarum, Aspergillus fumigatus, Candida albicans, Alternaria alternate – 11 (24%) was revealed. Only in 6 (13%) patients we cannot established the allergy specific IgE.

In parallel, the data of aeropolinometer Burkard Trap were defined in accordance with the calendar for distribution of aeroallergens reflecting concentrations of blossoming tree-plants and atmospheric aerosols in the air at a given period of time in Imereti region.

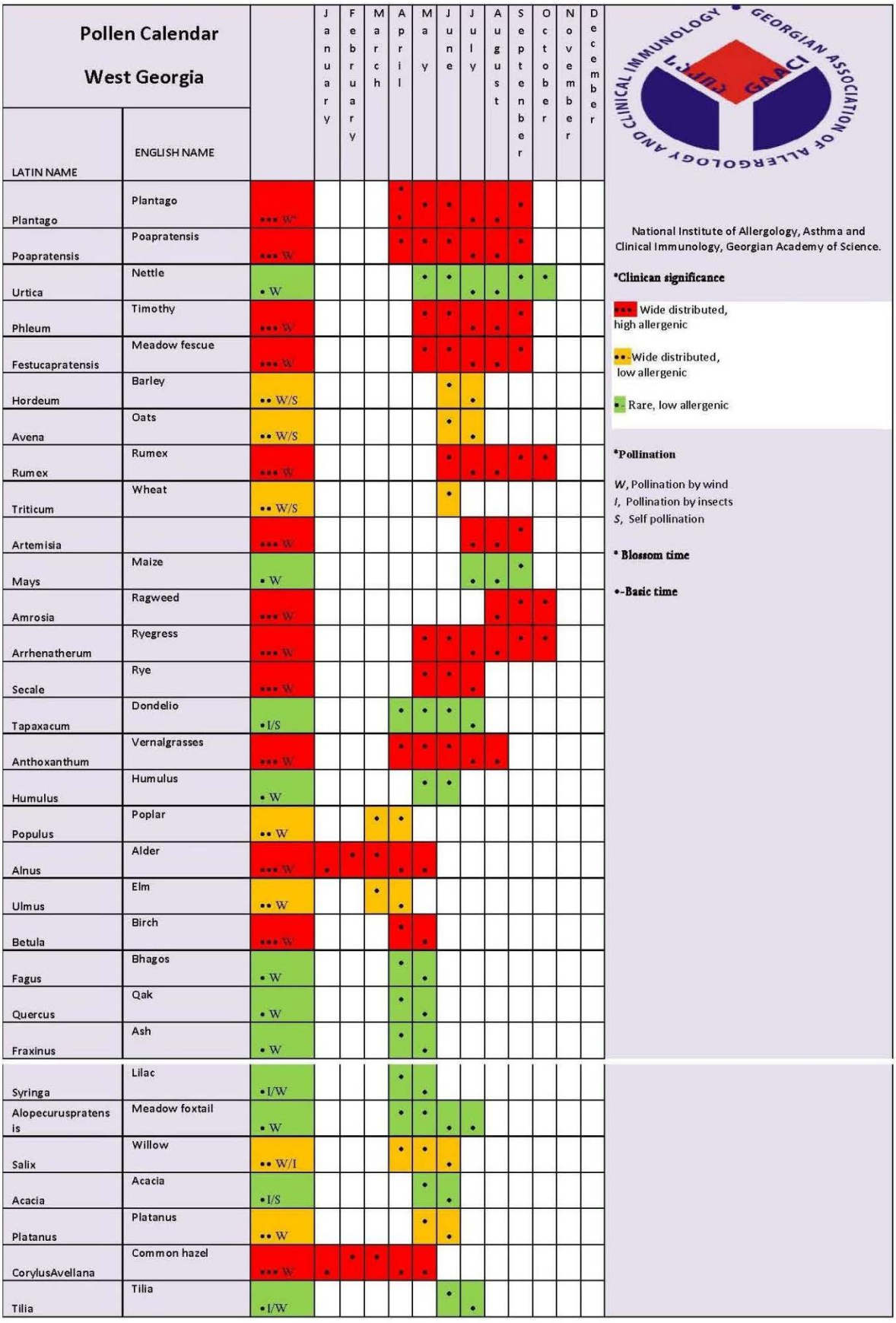
Permanent monitoring of the aeropolinometer Burkard Trap data proved that in west Georgia the beginning of aeroallergens' increasing concentrations is in March and the ending – in October, respectively, the peak is in May, when atmospheric concentrations of approximately 21 plants are increasing in the air simultaneously. Ambrosia, plantain, common hazel, birch tree, alder, ryegrass, flowering period of which is given in the calendar, are widely spread and characterized with high allergenicity.

Statistical analysis also confirmed direct-proportional increase admission of the patients with bronchialashmato our clinic with respect to the elevation of aeropollutants concentration in air. In addition, the statistical analysis proved proportional grows of applying patients with bronchial asthma to our clinic in relation to increasing concentrations of air pollutants in the air.

From January to April 2017, there were revealed a high concentration of aeropollutants, by high allergenization and widespread; especially high concentrations were found in alder, birch tree and common hazel, while from aeropollutants of low allergenization poplar, elm, willow and plane tree were distinguished.

Every above-mentioned diagnostic markers have high degree correlation with each other and correlation coefficient was r=0.8, on average. Calendar for distribution of aeroallergens in West Georgia, and updated information about the concentration of aeroallergens are constantly given to the patients who are being treated and/or registered. The study allowed us to provide the following conclusions: elevated concentration of Immuno CAP/Phadiatop in blood as well as total IgE high level proves existence of atopic allergy to inhaled allergens.

A concentration of different types of tree-dust and surrounded atmospheric aeropollutants was specified by using aeropolinometer Burkard Trap at a given period of time and consequently, the annual calendar for distribution of aeroallergens in West Georgia was developed over again. High degree correlation between the above-mentioned markers proves its clinical importance/value with respect to bronchial asthma.



ISSN 1562-3629

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Announcement on the retraction of the articles of scientific and periodic journal "International Journal on Immunorehabilitation"

At the request of the authors and on the basis of the decision of the editorial board of the “International Journal on Immunorehabilitation”, there are withdrawn the articles:

R.I. Sepiashvili “Immunorehabilitology: From immunotherapy to personalized targeted immunorehabilitation.” // *International Journal on Immunotrehabilitation*. 2016, 18(2): pp. 65–71.

Объявление об отзыве статьи из научно-периодического журнала "International Journal on Immunorehabilitation"

По просьбе авторов и на основании решения редакционной коллегии журнала “International Journal on Immunorehabilitation”отзываются следующие публикации:

Р.И. Сепиашвили «Иммунореабилитология: от иммунотерапии к персонализированной таргетной иммунореабилитации. // *International Journal on Immunotrehabilitation*. 2016, 18(2): pp. 65–71.

International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

NEW TRENDS IN THE DEVELOPMENT OF ANTIHISTAMINES AND IMMUNOACTIVE DRUGS: POSSIBLE ROLE OF HISTAMINE H3/H4 RECEPTORS AS A TARGET FOR NEW CLASSES OF DRUGS

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Histamine plays ат important role in the pathophysiology of allergic diseases, asthma, rhinitis and urticaria. The goal of this study was to investigate the different immune activities of a dual antagonists of H_3 and H_4 histamine receptors and on the possible role of a new types of histamine receptors as targets for the development of a new classes of antiallergic, immunoactive drugs.

Key words: *histamine, allergic diseases, asthma, rhinitis, urticarial, neuromediators*

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UDC 16-097:615.37

Histamine plays a prominent role in the pathophysiology of allergic diseases, asthma, rhinitis and urticaria [1, 2]. In human pathology, histamine triggers acute symptoms via its very rapid action on vascular endothelium and bronchial smooth muscle cells, leading to the development of acute symptoms such as nasal discharge, nasal congestion, bronchoconstriction, abdominal cramps, diarrhoea or skin wheal and a flare response.

The role of histaminergic neurons, their function and relevance are not well established. Except of central nervous system, H_3 receptors are involved in many processes outside of it, for example mast cell–neuron loop in the tissues, where H_3 receptors regulate intensity of neurogenic inflammation [3]. Histamine H_3 receptor and its antagonists may have clinical applications in treatment of diseases induced or influenced by the action of histaminergic neurons, thus leading to limited availability of certain neuromediators. Histamine is a mediator of a wide range of physiological and pathophysiological reactions in l organisms, one of the most important biogenic amines, often modulating the mechanisms of immunoregulation [2].

Biological effects of histamine are realized through specific signaling pathways represented by four known types of G-membrane receptor ligands (H_1R, H_2R, H_3R and H_4R). After the discovery of histamine receptors of types 3 and 4, it was found that the main role in regulating the growth and differentiation of hematopoietic progenitors under the influence of histamine still belongs to H_4 receptors [3]. Subsequently, it was proved that many of immune cells, like macrophages, dendritic cells e.c. express all 4 known types of histamine receptors [4, 5]. However, homologous to H_3 receptors, H4 subtypes have a significantly greater affinity for histamine compared to H_1 and H_2 receptors [3, 6, 7].

The last investigations demonstrated that all of the histamine receptors may be an effective targets for a new drug classes. The goal of this study was to investigate the different immune activities of a dual antagonists of H_3 and H_4 histamine receptors and on the possible role of a new types of histamine receptors as targets for the development of a new classes of antiallergic, immunoactive drugs.

Materials and Methods

Peripheral blood mononuclear cells (PBMC) of 10 healthy donors were cultivated in the presence of different dual $H_3/_4$ histamine receptor antagonists. The concentrations of cytokines, chemokines and growth factors in 48-hour supernatants of PBMC and DCs were assessed by Multiplex assays using Luminex xMAP technology. Supernatants obtained after 48-hours of cultivation in RPMI 1640-medium were assessed for the multiple cytokines, growths factors and chemokines (45-plex) by Multiplex assays using Luminex xMAP technology. Total IgE level of PBMC supernatants in 7-days culture was assayed by Phadia CAP method. The concentration of the key IgE-regulatory cytokines in PBMC supernatants were assessed by ELISA.

$H_3/_4$ histamine receptor antagonists with different affinity were kindly provided by Prof. W. Schunak (Germany).

 ISSN 1562-3629

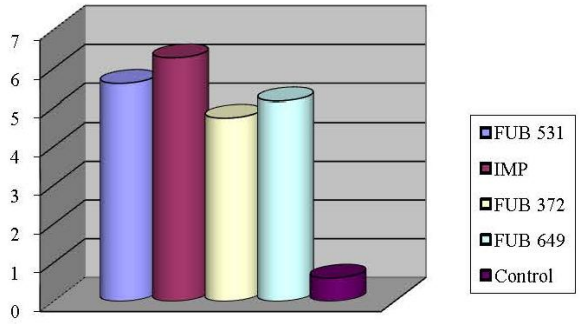

Fig. 1. The influence of H_3/H_4 antagonists (10^{-5}M) with different affinity on histamine-induced IgE synthesis.

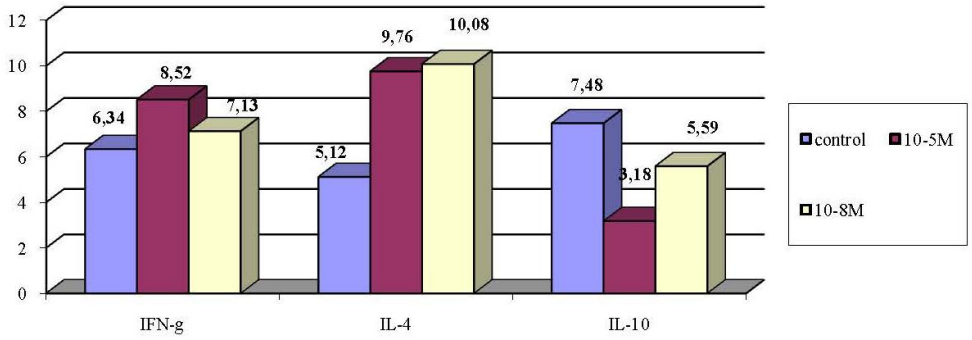

Fig. 2. The influence of Ciproxifan on the IgE regulatory cytokine synthesis by PBMC of of ragweed sensitive patients during remission.

Table 1

An influence of $H_3/_4$ antagonist (Ciproxifan) on the chemokine secretion by PBMC of healthy donors (n=10) in 48-hours culture

Chemokine	*Concentration (pg/ml; M±σ)*	
	Spontaneous	*Ciproxifan*
Eotaxin	6.8±0.73	13.2±1.95**
RANTES	140.8±45.59	710.3±426.51**
MCP-1	4663.4±1020.0	5283.5±1512.27
MIP-1 alpha	994.8±287.1	2293.4±1211.3*
MIP-1 beta	4812.2±2224.18	10451.7±7869.1**
GRO-alpha	533.4±135.48	2062.2±1087.94**

Results and discussion

In preliminary studies it was demonstrated the decrease in IgE synthesis by PBMC of healthy donors cultivated in the presence of histamine. Further experiments demonstrated that dual $H_3/_4$ antagonists may have an impact on this effect of histamine. It have been shown that dual $H_3/_4$ antagonists with different affinity cancelled (Fig. 1) the decrease of IgE synthesis modulated by high concentration of histamine (10^{-5}M).

This effect maybe due to the influence of $H_3/_4$ histamine receptor antagonists on the synthesis of |IgE regulatory cytokines. It have been demonstrated that Ciproxifan – one of the potent $H_3/_4$ antagonists dose-dependently increase the synthesis of γIFN and IL4, but decrease and IL-10 production by PBMC of healthy donors and ragweed sensitive patients (fig. 2) and this effect was statistically relevant ($p<0.05$).

Further experiments demonstrated the impact of H3/H4 histamine receptor antagonists on the synthesis of multiple cytokines, chemokines and growth factors.

It have been shown that cultivation of PBMC of healthy donors with H3/4 antagonist induce opposite effects on the secretion of cytokines and growth factors. H3/4 antagonist Ciproxifan significantly increases the secretion of such cytokines as ILβ, IL-4, IL-13, IL-18, IL-27 and IP-10). At the

same time it induces a noticeable inhibition of the secretion of SCF, GM-CSF, LIF, IL-2, IL-5, IL-6, IL-7, IL-9, IL-15 and IL-31.

In the series of experiments demonstrated that inhibition of H3/4 receptors by Ciproxifan resulted in a significant increase (table 1) in the synthesis be PBMC MOST of the main chemokines (Eotaxin, RANTES, MIP-1a, MIP-1b, GRO-alpha).

Thus, the study demonstrated that histamine dual $H_3/_4$ antagonists may have an important impact and clinical relevance not only on the central brain mechanisms, but also on IgE synthesis and secretion by PBMC of a multiple cytokines, chemokines and growth factors. This suggests that H_3 and, especially, H_4 histamine receptors are the effective targets for a new classes of antiallergic, immunoactive as well as anti-inflammatory drugs.

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Meeting Calendar

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December 6–9, 2018 Florence, Italy

WAO WISC MEETING

♦

February 22–26, 2019 San Francisco, USA

ANNUAL MEETING OF THE AMERICAN ACADEMY OF ALLERGY, ASTHMA AND IMMUNOLOGY (AAAAI)

♦

ISSN 1562-3629

International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

MAGNETIC NANOPARTICLES MODIFIED WITH SCFV MINI-ANTIBODIES FOR HER2/NEU-OVEREXPRESSING CANCER CELLS TARGETING

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Here we describe the design of multifunctional nanostructures based on genetically engineered recombinant mini-antibodies that selectively recognize oncomarker HER2/neu on the human breast adenocarcinoma cells SK-Br-3. ScFv mini-antibodies, offer significant advantages over full size antibodies for targeted delivery of nanoparticles both *in vitro* and *in vivo*, such as low cross-reactivity and immunogenicity and ease of biotechnological production in prokaryotic expression systems. The nanostructures were obtained through the use of the proteinaceous barnase*barstar interface between nanoparticles and recognizing antibodies. Moreover, for demonstrating the universality of this approach for creating scFv and magnetic nanoparticles-based constructions, we obtained complexes of magnetic nanoparticles and fusion protein of anti-HER2/neu mini-antibody 4D5scFv and fluorescent protein mCherry that selectively stained HER2/neu-positive cells. Application of such targeted nanoparticles opens up new possibilities for designing new effective methods of cancer diagnostics.

Key words: *HER2/neu; barnase; barstar.*

Abbreviations used: EGFR: epidermal growth factor receptor; HER2/neu: human epidermal receptor 2; MP: magnetic particles; scFv: single chain variable fragments.

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UDC 57.089:621.318

Currently, advances in nanobiotechnology open up new possibilities for diagnostics and treatment of wide range of different diseases by essentially new means and ways [1–7]. Nanoparticles, possessing fundamentally different properties as compared to bulk samples and small molecules, attract a particular attention of researches as a unique tool for solving a certain spectrum of biomedical problems. Moreover, a number of nanoparticle-based therapeutics has already been approved for treatment purposes [8]. Combination of diagnostics and therapy of various diseases, known as theranostics is of particular interest for modern biomedicine. There are a lot of theranostic nanoparticle-based platforms, but among them, magnetic particles (MP) are of special importance due to their unique physical nature and diverse functionality. A possibility to manipulate by MP with external magnetic field allows one to locate them in a certain area, like a particular tissue of the organism, and MP are suitable for hyperthermia of tumors since they can be inductively heated with an AC magnetic field. One of the important properties of MP as agents for theranostics is their ability to be surface-functionalized by various molecules ensuring biological activity of the particles; among these molecules are toxic and/or visualizing modules, as well as targeting agents for specific particle delivery to a certain cell type.

For the targeted delivery of MP to cells, full-size antibodies immobilized on the MP surface by various means are successfully employed. In some cases, however, a more perspective approach for this purpose is the use of molecular constructions based on the antigen-binding sites of the antibody, such as, for example, mini-antibodies of the scFv format (single-chain variable fragments), consisting of the variable domains of light and heavy chains of immunoglobulin connected with a flexible peptide linker [9]. A small size (25–30 kDa) and the absence of the constant domain reduce the immunogenicity of such molecules. It should be also noted that biotechnological production of such structures in prokaryotic expression systems is relatively easy as compared to full-size antibodies; besides, more elaborated fully genetically encoded constructions can be developed with mini-antibodies, containing, for example, toxic or visualizing modules [10].

MP equipped with targeting molecules are also prospective objects for a variety of immunoassays both *in vitro* and *in vivo*. Selectively delivered MPs within the body allow carrying out non-invasive immunoassay by means of MP localization detection by an external induction probe. Similarly, such targeted particles could be used for quantitative detection of specific molecules in biological samples which can serve as an alternative method to some of the existing methods of disease diagnostics.

Here, we describe MP-based constructions containing genetically engineered recombinant mini-antibodies that selectively recognize oncomarker HER2/neu on the surface of human breast adenocarcinoma SK-Br-3 cells. HER2/neu, a receptor belonging to the epidermal growth factor receptor (EGFR) family, is overexpressed in about 30% of the human breast carcinomas and in many other types malignant tumors in human [11]. HER2/neu amplification was observed in prostate carcinoma, malignant tumor of endometrium, stomach primary cancer, ovary and renal cancer [12]. HER2/neu overexpression often correlates with drug resistance, and high metastatic potential of the tumor, as well as with high risk of recurrence and a poor survival prognosis. Thus, early and precise detection of the HER2/neu expression on the surface of cancer cells is of high importance for clinical diagnostics.

Materials and Methods

MP Synthesis and Conjugation. Carboxymethyldextrane-coated MP were synthesized as described previously [1–2]. MP containing carboxylic groups on the surface, were covalently bound with protein molecules using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC, Fluka) as a crosslinking agent with a formation of a stable amide bond between the amino group and carboxyl group on the MP surface. The reaction was carried out in two stages: first, MP were activated with EDC in 0.1 M solution of 2-(N-morpholino)-ethane sulfonic acid, pH 5.0, and then, after the removal of the EDC excess by centrifugation, protein was added in the phosphate buffered saline (PBS, pH 7.4). The MP suspension was periodically sonicated to prevent the MP aggregation during the reaction. Conjugation lasted for at least 2 hours; the unreacted protein was removed by centrifugation. Hydrodynamic radius and ζ-potential of the obtained conjugates were determined using Zetasizer Nano ZS analyzer (Malvern Instr.)

Isolation and Purification of the Proteins. Isolation and purification of proteins 4D5scFv, 4D5scFv-Bn-4D5scFv, and barstar were performed as described in [13], [3], and [14], respectively. To produce the 4D5scFv-mCherry protein, *E. coli* SB536 strain was transformed with pSD-4D5scFv-mCherry and grown in LB at 28°C. At OD550=0.8 the culture was induced with 1 mM IPTG and then incubated at 28°C for 15 h. Purification of 4D5scFv-barnase-4D5scFv fusion protein was carried out on "HisTrapFF 1 ml" column (GE Healthcare), with subsequent purification on "HiTrap Protein A HP 1ml" column (GE Healthcare), according to the manufacturer procedure. The protein homogeneity was confirmed by SDS-PAGE analysis in 12.5% polyacrylamide gel according to the standard protocol.

Cell Culture. Human breast adenocarcinoma cells SK-Br-3 and Chinese hamster ovary cells CHO were cultured in RPMI-1640 medium (HyClone) supplemented with 10% fetal calf serum (HyClone) and 2 mM L-glutamine (Pan-Eco) at 37°C under a humidified atmosphere with 5% CO_2. The cells were passaged 2–3 times a week.

Fluorescent microscopy analysis. Cell suspension at $2.5 \cdot 10^4$ cells/ml was seeded into 96-well plates and cultured overnight. Before the experiment the cells were washed with PBS and cooled down to 4°C. Then, the studied MP conjugates in 3% fat-free milk were added to the cells and incubated for 45 min at 4°C. After that, the cells were washed thrice with PBS and analyzed with the inverted fluorescent microscope Axiovert 200 (Carl Zeiss).

Analysis of MP Binding with Cells. Samples for the detection were prepared as follows. The cells were seeded into 24-well plates and cultured overnight. Before the experiment the cells were washed with PBS and cooled to 4°C. To assess the interaction of the cells with MP directly bound to antibodies, the cells were incubated with conjugates in PBS supplemented with 1% of bovine serum albumin (BSA) for 45 min. Then the cells were washed with PBS thrice to remove non-bound conjugates. For estimation of MP binding with cells our original MPQ-cytometry method was used [2, 15]. For two-step labeling, the cells were first incubated with protein 4D5scFv-Bn-4D5scFv in PBS for 30 min, then washed with PBS, and further were incubated with MP-Bs conjugates in PBS with 1% BSA for 30 min and thrice washed with PBS. Following the incubation with MP sample, cells were harvested from wells' surface with 2 mM EDTA solution, centrifuged one time and magnetic signal of a pellet resuspended in 30 μL PBS was measured. The obtained signal was normalized to the cell number in a sample, calibrated accordingly to the actual quantity of magnetic material in a sample and presented as picograms of MP per cell.

Results and Discussion

As a targeting molecule for specific delivery of MP to the cell surface mini-antibody 4D5scFv capable of binding with extracellular domain of the HER2/neu receptor was used [16–17]. For investigation the binding specificity of the obtained conjugates with the HER2/neu receptor, human breast adenocarcinoma cells SK-Br-3 were used. This cell line is known to overexpress HER2/neu in the amount of ~10^6 receptors per cell, while in normal cells the expression does not exceed 10^3–10^4 receptors per cell and may be absent at all [18]. To control the binding specificity, Chinese hamster ovary cells (CHO) were chosen, as a line which does not express none of the EGFR family receptor [19].

Conjugates of MP with 4D5scFv obtained by carbodiimide method appear to non-specifically bind with control CHO cells; besides, the conjugated particles exhibited poor aggregation and sedimentation stability. It is probably caused by the fact that 4D5scFv is a small positively charged protein at pH=7.4 (calculated pI value is 8.5) and upon conjugation with this protein, on the surface of particles local positive charges are formed that stimulates aggregation of MP having negative charge on their surface. Indeed, ζ-potential of MP after conjugation increased from –18.9±0.8 mV to –12.1±0.7 mV; MP size also increased considerably, suggesting particle aggregation (Table 1). The problem of the MP–4D5scFv specific binding with cells may also emerge due to steric hindrance (small size of the

ISSN 1562-3629

mini-antibody) upon recognition of the antigen on the cell surface. Besides, one protein molecule can bind with the MP surface through several amino groups; this may lead to partial denaturation of the molecule and a loss of its functional activity.

To design MP that specifically recognize oncomarker HER2/neu on the cell surface, we employed a barnase*barnstar protein system. Barnase (Bn), a bacterial ribonuclease of *Bacillus amyloliquefaciens*, and its natural inhibitor barstar (Bs) are small proteins (12 and 10 kDa, respectively) with extremely fast kinetics (k_{on}~ 10^8 $M^{-1}\cdot s^{-1}$) and high binding affinity of binding (K_a~ 10^{14} M^{-1}) [20]. Conjugation of one of the proteins from this pair with MP and combining the other one with mini-antibodies 4D5scFv allow creating structures that specifically recognizing HER2/neu on the cell surface (see Fig. 1A). This approach also allows one to perform a two-stage delivery of nanoparticles to the cells with creating of functionally active complex when necessary [20].

Earlier, the barnase*barnstar system was successfully used by us for construction of various fully genetically engineered constructions with fluorescent proteins [21], complexes with quantum dots [20], nano-diamonds [22], gold and magnetic particles, as well as hybrid structures combining several elements [3–4], which have a potential application for diagnostics and therapy of oncological diseases.

For conjugation with MP we chose barstar and for the specific two-stage delivery of the MP-Bs, fusion protein 4D5scFv-Bn-4D5scFv consisting of two molecules of mini-antibody 4D5scFv and one molecule of barnase was used. The choice of barstar for conjugation with the MP surface was justified as follows. Under neutral conditions barstar is negatively charged, as pI (Bs)=4.6; therefore, upon conjugation with MP this protein should prevent particle aggregation that can be caused by neutralization of their surface charge. Indeed, MP-Bs conjugates did not aggregate; after conjugation particle size and ζ-potential remained virtually unchanged (Table 1). Functional activity of barstar on the MP surface was assessed by ζ-potential measured after the incubation of MP-Bs conjugates with 4D5scFv-Bn-4D5scFv; an increase in the particle ζ-potential confirmed binding of the conjugates with fusion protein (Table 1).

The quantity of MP bound to the cells was estimated by an our original MPQ-cytometry method, as described in Materials and Methods section [2]. This technique designed for detection of magnetic materials allows a highly sensitive real-time detection in dynamic range up to seven orders of magnitude at room temperature. This method has been successfully applied for various measurements both *in vitro* (in immunoassays using magnetic particles as markers of biochemical reactions) and *in vivo* (for studies of the MP behavior in the organism of experimental animals) [15, 23–24].

The cells were pre-incubated with 4D5scFv-Bn-4D5scFv, then the excess of the non-bound protein was removed, and the MP-Bs conjugates were added. Fig.1A illustrates the interaction between the obtained MP conjugates with the cells. The ratio of the MP number bound with SK-Br-3 cells to the MP number bound with control cells CHO is 8.3±0.7. Thus, the barnase*barstar module helped to obtain colloidally stable conjugates of MP with mini-antibodies of the scFv format that specifically recognize tumor cells overexpressing oncomarker HER2/neu.

Among the advantages of mini-antibodies is the option to generate fully genetically encoded constructions with targeted action for tumor cell visualization. Combining of such constructions with MP allows creation of multifunctional agents for visual and quantitative estimation of the expression of certain antigens on the cell surface, which may be useful for diagnostic purposes.

We have obtained colloidally stable conjugates of MP with fusion protein 4D5scFv-mCherry; their hydrodynamic size and ζ-potential are given in Table 1. MCherry is one of the most popular proteins of the mFruits series that optimally combines maturation time, photostability and brightness as well as the fluorescence emission wavelength [25]. As pI (mCherry) =3.8 (calculated value), the mini-antibody 4D5scFv as a part of this fusion protein did not induce MP aggregation upon conjugation and retained its functional activity. These conjugates MP-(4D5scFv-mCherry) specifically stained the surface of the tumor cells overexpressing HER2/neu, as was visually demonstrated with fluorescence microscopy (Fig. 1B).

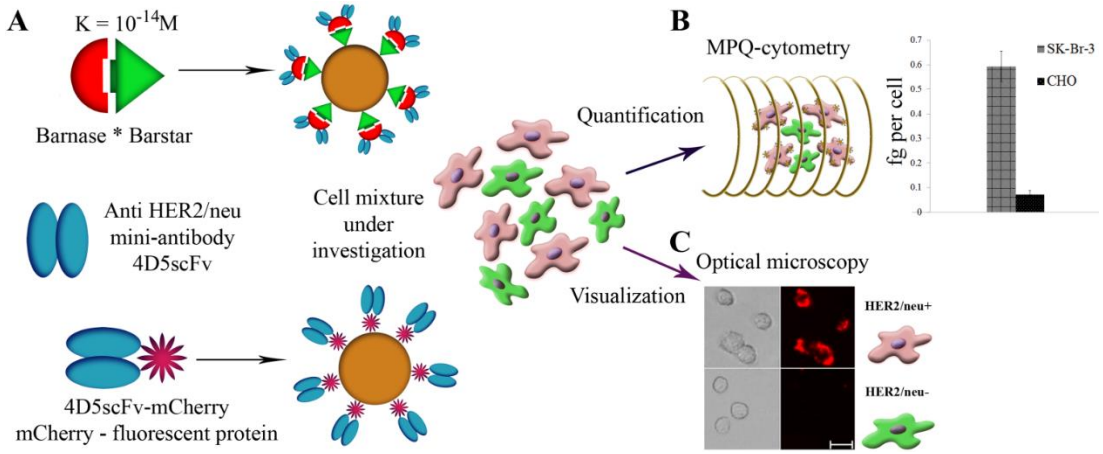

Fig. 1. **A.** Scheme of specific cell labeling. **B.** Quantity of structures (MP-Bs)*(4D5scFv-Bn-4D5scFv) bound with cells SK-Br-3 (overexpression of HER2/neu) and CHO (control cell line). **C.** Specific visualization of HER2/neu on cell surface with MP-(4D5scFv-mCherry) conjugates. I – SK-Br-3 cells, II – CHO cells, left – bright field images; right – fluorescent images. Scale bar, 20 μm.

Table 1.

ζ-porential and mean hydrodynamic diameter of magnetic particles and magnetic particle-based conjugates

Particle notation	*ζ-potential, mV*	*Radius, nm*
MP	-18.9±0.8	50.5±2.0
MP-4D5scFv	-12.1±0.7	92.3±1.2
MP-Bs	-18.2±0.5	46.9±0.6
(MP-Bs)*(4D5scFv-Bn-4D5scFv)	-15.2±1.1	46.5±1.1
MP-(4D5scFv-mCherry)	-15.5±0.3	48.2±0.3

In this work we have obtained constructions based on MP and recombinant mini-antibodies selectively recognizing oncomarker HER2/neu. High specificity of the binding of the obtained constructions with human breast adenocarcinoma cells was demonstrated both quantitatively and visually. Thus, we have demonstrated ample opportunities of applications of mini-antibodies and their genetically encoded derivatives as well as nanocomplexes on their basis for a number of biomedical tasks, in particular, for labelling and quantitative analysis of HER2/neu-positive cancer cells.

Acknowledgment. *The work was supported in part by the Russian Science Foundation grant №14-24-00106 (magnetic particles synthesis, proteins purification) and by the Russian Foundation for Basic Research and by the National Intellectual Development Foundation (NIDF) according to the research project №17-34-80105 "mol_ev_a" (nanoparticle modification, cell culture).*

The study was partially performed using equipment provided by the IBCH core facility (CKP IBCH, supported by Russian Ministry of Education and Science, grant RFMEFI62117X0018).

Authors acknowledge support from the MEPhI Academic Excellence Project (Contract No. 02.a03.21.0005).

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International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

PHOTODYNAMIC DESTRUCTION OF DEEP-TISSUE TUMORS USING BIOLUMINESCENCE RESONANCE ENERGY TRANSFER

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Photodynamic therapy (PDT) is a clinically proven, non-invasive strategy to treat tumors. Since PDT requires an external light source, and the penetration depth of visible light in tissues does not exceed 10 mm, PDT is effective only for the treatment of superficial tumors. To overcome this serious limitation, we propose to use a bioluminescence resonance energy transfer (BRET)-based PDT strategy, which does not require an external light source.

Key words: *bioluminescence resonance energy transfer, photodynamic therapy, NanoLuc luciferase, furimazine, hepatotoxicity.*

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UDC 616.5-006.621.316.172

Photodynamic therapy (PDT) is a minimally invasive cancer therapy that includes accumulation of a photosensitizer (PS) in the tumor and subsequent illumination with light of a specific wavelength [1–2]. One of the main problems of contemporary photodynamic therapy is delivery of therapeutic light to the deep-lying tumors and metastasis because of the light refraction, reflection and absorption by biological tissues. It is well known that the penetration depth of visible light does not exceed 10 millimeters in tissue [1]. To overcome the general defect of the method we propose to use bioluminescence as an internal light source.

The bioluminescent systems based on Förster resonance energy transfer are widely used in optical imaging and biomedical applications [3–4], but its potential for therapeutic exploration has been recognized only recently [5–9]. One of the promising research directions in this field is creation of quantum-dots (QD)-luciferase conjugates in combination with chemical PSs [8–9].

In our work we propose another way [10–11]. Using in one reading frame genes encoding phototoxic protein miniSOG (as a PS) [12] and NanoLuc luciferase (as a light source) [13] we have shown that NanoLuc bioluminescence system causes photodynamic effect comparable with conventional PDT. Proposed bioluminescence system possesses efficient energy transfer to miniSOG excitation and reactive oxygen species generation.

We discovered, that highly specific NanoLuc substrate, furimazine, possesses hepatotoxicity in mice, but splitting the therapeutic dose into several injections significantly reduces hepatotoxicity of furimazine *in vivo*.

Based on our data we can state that the BRET reaction with bioluminescence system NanoLuc-furimazine and genetically encoded photosensitizer miniSOG is a promising system for photodynamic therapy of deep tumors and can be considered as an alternative to external light sources for *in vivo* phototherapy.

Results and discussion

To test our approach, we designed three plasmids each expressing a NanoLuc-miniSOG variant targeted to either the cytoplasm, or mitochondria, or cell membrane. These vectors were used to produce stably transfected clones of human breast adenocarcinoma cell line SK-BR-3. These sublines were subsequently used to confirm the expression and intracellular localization of NanoLuc-miniSOG variants, to demonstrate BRET and cell toxicity *in vitro* (Fig. 1).

To study furimazine toxicity *in vivo*, the substrate was intravenously administered to mice at a dose of 20 μg/animal for 7 days, once, twice, or thrice a day. After a 7-day course of furimazine intravenous injections, a histology study of internal organs of mice was performed. Intravenous administration of furimazine caused the most pronounced toxic effect on liver: at all doses, a hepatotoxic effect was observed (Fig. 2).

The maximum damage was observed upon introduction of 60 μg furimazine per day, which led not only to hydropic dystrophy but also to hepatocyte necrosis. Upon introduction of 40 μg per day, the toxic effect was less pronounced and limited to hydropic dystrophy in lobule centers and in subcapsular regions. Administration of 20 μg per day caused minimal toxic effect, and splitting of the dose into three decreased the liver load considerably: almost no damage could be registered visually upon administration of 7 μg

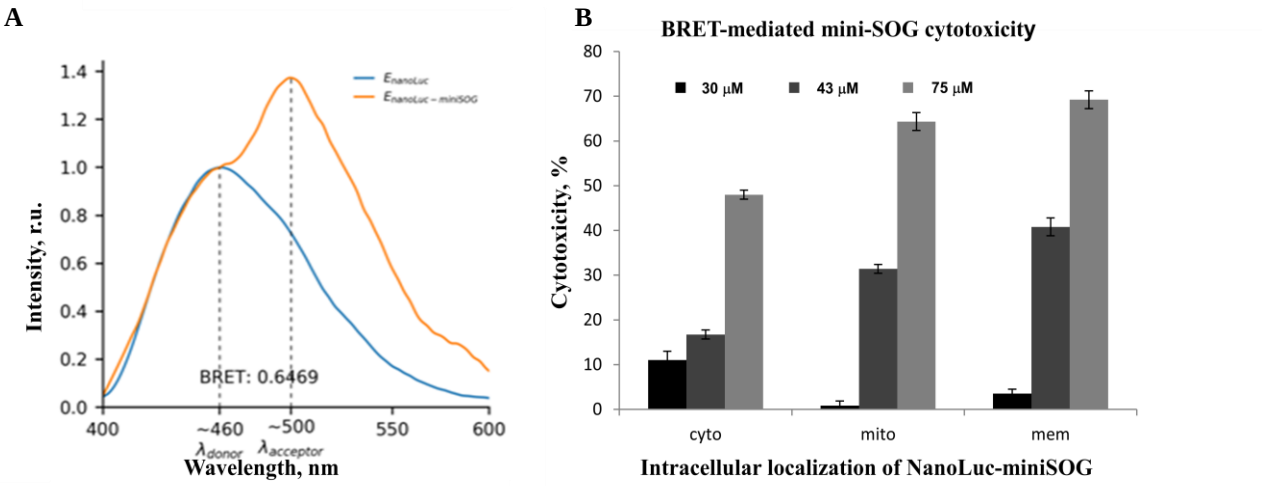

Fig. 1. *In vitro* characteristics of genetically encoded NanoLuc-miniSOG BRET-system. A – BRET-ratio calculation in NanoLuc-miniSOG system. The BRET coefficient is calculated as the ratio of the emission values of the NanoLuc-miniSOG system measured at the maximum acceptor emission wavelength to the same value measured at the donor wavelength, minus the same ratio determined from the donor spectrum alone. C – MTT assay of BRET-induced miniSOG phototoxicity.

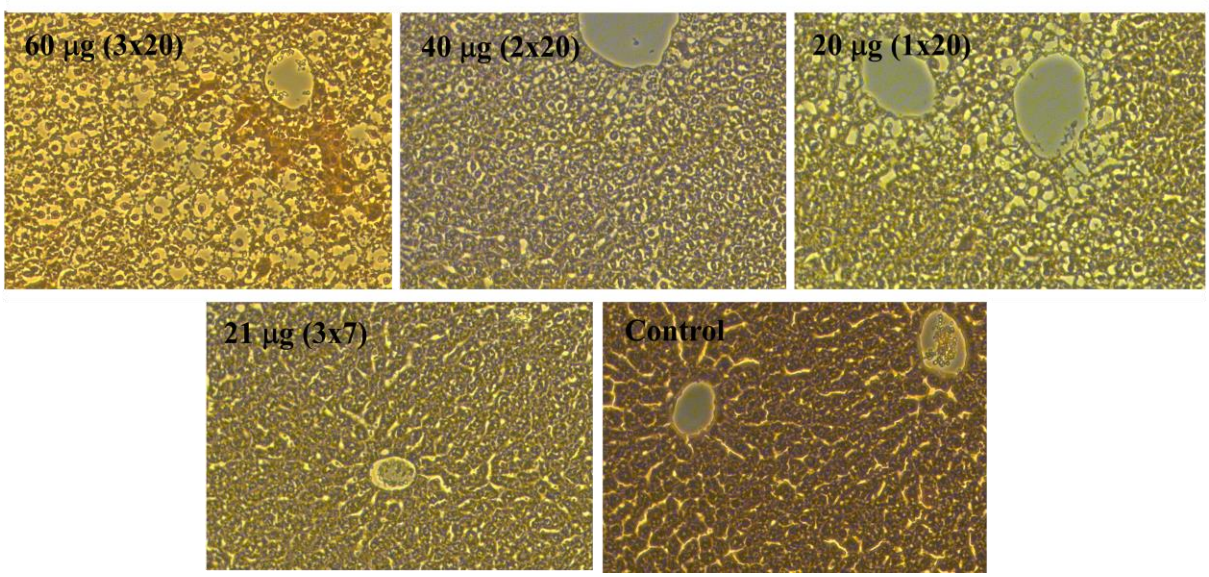

Fig. 2. Toxicity of furimazine *in vivo*. Histology analysis of liver sections of mice treated with various doses of furimazine. Dose per day is indicated at every image. Hematoxylin and eosin staining, ×100 magnification. The images were obtained with Zeiss microscope (Axiovert 200).

three times a day (Fig. 2). Therefore, the strategy of stepwise furimazine administration can be attempted for higher injection dose.

Conclusions

Being fully genetically encoded, our system has the following significant advantages over QD-luciferase conjugates and chemical PS: easy targeting to any cell compartment by fusing with localization motifs; expression can be controlled by tissue- or tumor-specific promoters; targeted gene delivery, for example, using pseudotype virus delivery systems, to cells carrying known onkomarker.

We believe that genetically encoded system for BRET-mediated PDT presented in this work may be a new paradigm of how to deliver light and photosensitizer in deep tissues and metastasis.

Acknowledgments. *This research was supported by RSF grant no 16–14–10321. The study was partially performed using equipment provided by the IBCH core facility (CKP IBCH, supported by Russian Ministry of Education and Science, grant RFMEFI62117X0018).*

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Meeting Calendar

International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

PROSPECTIVE IINNOVATIVE STUDIES ON THE DIAGNOSIS OF UROTHELIAL CANCER

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The urothelial cancer (UC) is one of the leading pathologies among urological cancers. A significant part in the development of cancers is taken by molecular genetic disorders, their research allows identify new trends in diagnostics and prediction of the disease. The article has comprehensively analyzed modern native and foreign literature based on the UC phenotype data, the results of the molecular pan-cancer analysis, new UC classification based on the investigations of the genetic profile of various forms of UC and brief review of molecular genetic markers of early identification and prognosis of how UC will run are presented.

Key words: *urothelial cancer, bladder cancer, urothelial carcinoma, diagnosis, molecular-genetic markers and biomarkers, cancer-testis antigens, prognosis, survival.*

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UDC 616.62-006.6-07-036.65

The urothelial cancer (UC) is one of the leading pathologies among urological cancers that cover 13% in the total mortality structure. In Russia, from 2006 to 2016 the number of UC patients has increased by 1.44 times or nearly 22 people per a population of 100.000. According to the experts' prognostication, the growing UC incidence rate is considered as "alarming", because approximately 7.6 million people die from this disease annually. Extremely scanty manifestations at early stages of the disease lead to its late identification. An insufficient informative value and limited abilities of currently used diagnostic methods have been noted, due to which their improvement as well as development of new methods and markers of early diagnostics and prediction of the disease with maximum specificity, receptiveness and informative value are ongoing. Basic research carried out during the last decade has been focused on the optimization of UC histological and genetic criteria.

Modern trends in UC diagnostics and prognostication

Currently two separate networks of genomic alterations related to the UC molecular variety which can reflect various ways of tumor growth: superficial surface (muscle-non-invasive) and muscle-invasive, have been identified. [1–2]. An extremely heterogenic genetic profile is a characteristic feature and one of the main problems of UC. 5 molecular types of UC have been identified [3]: Urobasal A (UroA), Urobasal B (UroB), GU, SCCL and an infiltrated subtype characterized by predominance of expression of non-tumour phlogistic transcriptases. The analysis of genome alterations typical for UC and their connections with the molecular subtypes has shown that Urobasal A and B subtypes are characterized by a loss of chromosome 9 with 1q boosting ratio. GU and SCCL subtypes are featured by complicated disorders with frequent focal genome alterations 6p22 (E2F3/SOX4). In the SCCL subtype, the male/female patient ratio is 1:1, which indicates UC with the keratinized/planocellular phenotype develops more frequently [4]. Two main UC genome patterns have been identified: FGFR3/CCND1 chain, functioning in Urobasal tumours, and E2F3 / RB1 chain in GU tumours. For the SCCL subtype the chain has not been identified. The molecular Uro, GU and SCCL subtypes are featured by a high risk of progressing [2–3]. Based on genetic aberration studies, three UC subgroups have been identified: basal, luminal and highly differentiated intraluminal (similar to breast cancers) ones [5].

Molecular genetic research

A significant part in the development of cancers is taken by molecular genetic disorders, their research allows identify new trends in diagnostics and prediction of the disease. At that, the researchers focus on DNA methylation analysis; genetic networks of lipid metabolism regulation; RNA role; FOXM1 and PLK1, FOXM1, PPARG, FOXA1 and GATA3 protein expression levels; basal keratin expression, etc. [6]. The data obtained allowed carrying out a molecular pan-cancer analysis based on which SCCL was combined in the same group with flat lung, head and neck cancer [7–8]; four tumor clusters (clusters I–IV) which express genes typical for epithelial cells including KRT14, KRT5, KRT6 and EGFR, have been identified [7].

 ISSN 1562-3629

Three main molecular ways, which most suffer at UC [6]: regulation of the cell cycle (93%), kinase and PI3K (72%); remodeling of chromatin, including gene mutations (89%); SWI/SNF nucleus remodeling components (64%), have been identified.

It has been established that FGFR3 and PIK3CA (a catalytic subunit of PI3-kinase α) mutations play a key role for muscle-invasive tumors; it has been noted that in approximately 20% cases activation of genes of the RAS (HRAS1, KRAS and NRAS) family can be seen and takes place as a result of point mutations in 12, 13 and 61 codons of these genes [3, 9].

In situ cancers are characterized by mutation of tumor growth suppressor genes – TP53, RB and PTEN. For muscle-invasive UC, damages of tumor growth suppressor genes p53 (TP53), RB1 and PTEN and their inactivation and deletion by way of anomalous methylation of promoter regions have been noted [4].

The invasion and tumor grade have been determined by genetic alterations (Fig.1).

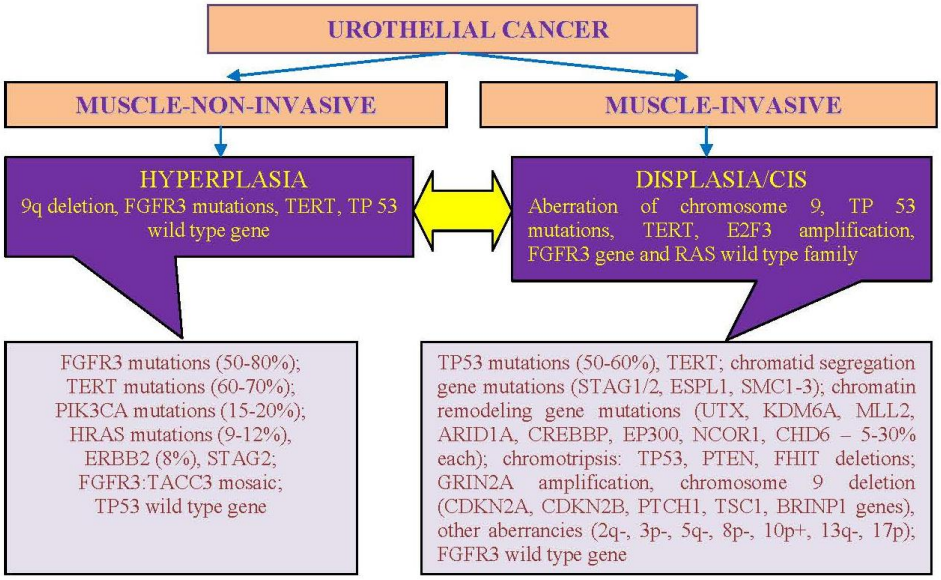

Fig. 1. Genetic mutations identified in UC patients with various degrees of tumor invasion.

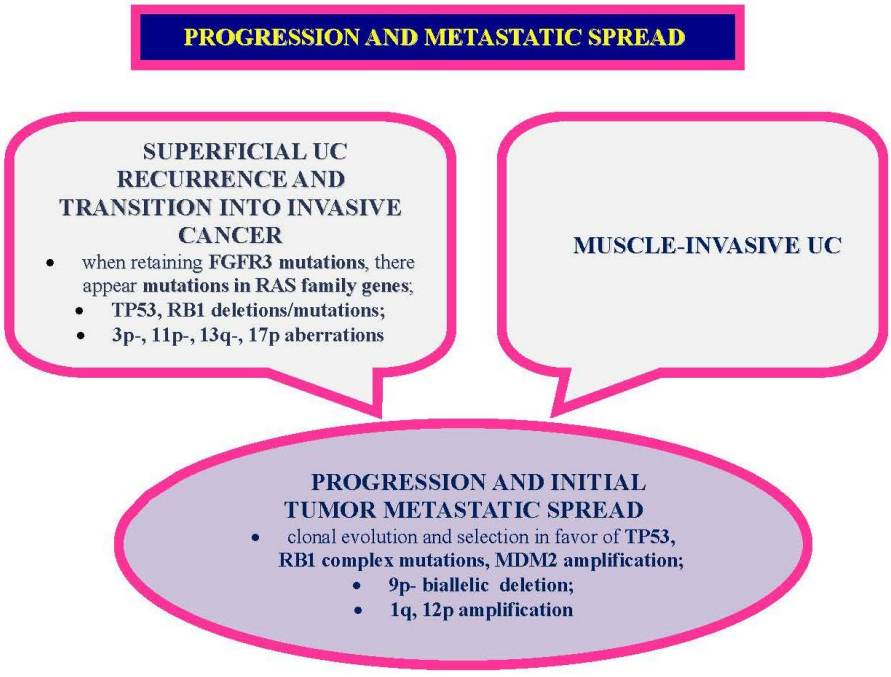

Fig.2. Relation of gene mutations to UC progression and metastatic spread.

Minimum manifestations can be identified at muscle-invasive tumours – monosomy of the 9th chromosome and 9p and 9q deletion alongside with 8p, 17p, 13q, 11p and 14q (30–60%) deletions. Muscle-invasive cancers are featured by p53 mutations and 17p, 3p, 13q, 18q or 10q locus heterozygosis losses [5] which are more frequently identified in low differentiated tumors and at later stages [10]. We have shown [11–12] that all tumor cultures had molecular genetic alterations of the cell karyotype typical for UC: deletion of the 9th chromosome (66.7%), lack of Y-chromosome (50%) and monosomy of the 13th and 17th chromosomes (33.3%). In individual cases, alterations in chromosomes 1, 3, 7 and trisomy 7 syndrome were registered. Intensification of alterations with stages of the disease, the morbidity rate and degree of malignancy were noted. A possibility of applying genetic alterations typical for various stages of UC as additional factors for prediction of UC clinical progression and metastatic spread has been studied [12–13]. The loss of 17p heterozygosis (locus of p53 gene) can be found in 60% of cancers with invasion into the muscle layer and thus can be a marker of progression (Fig.2).

A comparative analysis of the level of expression of genes and proteins of tumor and normal cells with survival of patients has shown that cancer cells are characterized by a higher expression of genes participating in DNA replication, cell fission and programmed cell death. For UC BLCAP (HGNC Symbol) gene, encoded protein, decreasing cell expansion and stimulating apoptosis, have been identified. Cancer Genome Atlas has described F0XA1 mRNA gene and protein, which are expressed on UC tumor cells [5]. The differentiated alteration of mRNA expression distinguishes the normal tissue from the tumor one, the invasive UC from its cell-surface form. The research carried out has shown that mRNA-141 and mRNA-205 were associated with overall survival [12, 14]. A certain success has been achieved in the area of studies of long, non-encoded RNA (dnRNA) and MALAT1. A connection between MALAT1 and development of tumor metastases has been shown. In addition to this, a role of aberrant expression of MALAT1 at UC has been demonstrated. Blocking of MALAT1 in a tumor can be efficient for preventing tumor metastasis development [10].

Markers of early diagnostics and prognostication

An important section of diagnostics is a skill to timely prognosticate occurrence of various diseases, including cancers, and that means the availability of instruments (methods and markers) in doctors' hands for their early diagnostics. It appears that identification of markers which could be recommended for widespread use in clinics for prediction of the outcome of the disease, its progression and metastatic spread, as well as markers allowing prediction of an aggressive potential of non-invasive tumors, is of special importance. Due to this, many researchers continue studying a possibility to practically apply molecular-genetic and molecular-cytogenetic markers. In particular, for UC a potential of their application as additional factors to determine prediction of clinical progression and early identification of metastatic spread process [13, 14], as well as a choice of optimum treatment schemes [15–16], are investigated. Among diagnostic markers proposed today only six of them have been approved and are used in Europe and America for early identification of UC: BTAStat, BTA TRACK, NMP-22, BladderChek, ImmunoCyt and UroVision. In Russia, markers are not actually used for regular diagnostics. A significant number of markers are at the stage of development and research. They include: determination of levels of cytokeratins 8, 18 (UBC), 19 (CYFRA21.1), 20 (CK20); hyaluronic acid and hyaluronidase; fibronectin; DD23; anti-apoptopic molecules (BCLA-4, Survivin); telomerase (TRAP, hTert, hTR) activity; hyper-methylation of promoter regions of RASSF1, RARB, p16, p14, CDH1 genes and a micro-satellite analysis as a method of identification of allelic imbalance. Some researchers have shown [17] that more than 90% UC are positive in reaction with CK7; CK20 co-expression has been noted according to the data of various researchers in 30 to 90% cases [18–19].

The most investigated group of UC markers is regulators of cell cycle (p53, Ki-67, pRb, mdm2). p53 (tumor suppression regulator) is researched as an independent predictor of tumor progression and backset; Ki-67 is considered as a factor of proliferative activity. However, its prognostic value hasn't been universally confirmed.

Recently, calcium activated regulators of chloride duct (CLCA) that participate in cell differentiation, adhesion, apoptosis, tumor progression and inflammatory process development have been researched as new markers [20]. Other researches show a possibility of using growth-stimulating factors and their receptors as prognostic markers. Thus, for example, tumor associated macrophages (TAMs) play a key role in tumor proliferation, invasion and metastatic spread [18–19].

In solid tumor immunogenesis, an important role is played by angiogenesis in which a key role is played by the vascular endothelium growth factor (VEGF). Apparently, the VEGF and HER2/neu expression can be UC prediction markers. Reduction of expression by the cell adhesion molecule tumor (E-cadherin, beta-catenin, ICAM-1, VCAM-1, selectins, integrins, bridge corpuscles) promote invasion and tumor metastatic spread [4].

The level of carcinoembryonic proteins IMP3, glypican-3 and TPBG authentically correlate with recurring and mortality. The research of tumor-associated testis-specific – NY-ESO-1, MAGE-A3, LAGE-1 and PRAME has shown an authentic correlation of their expression both with the disease stage and the invasion stage that can play a key role in determination of UC treatment tactics [11, 18, 21].

Currently, much attention is given to researching control protective points, the so called "check-points" – CTLA4 (cellulotoxic T-lymphocytic antigen 4), B7-H3 (CD276) [20]. The PD-1 (programmed death 1) receptor, as well as its PD-L1 ligand, are also researched as UC target markers. [8]. The tumors expressing PD-L1, through interaction of the PD-1 inhibiting receptor, can turn cytotoxic-educated cells into inactivated ones [22].

Apart from this, expression by PD-L1 tumor may indicate patients for timely treatment [23]. Individual investigations of GD2 ganglioside role in UC have shown a high level of GD2 expression at muscle-invasive UC [9].

Of certain potential are studies of cancer-associated testis-specific antigen complex: NY-ESO-1, MAGE-A3, LAGE-1 and PRAME for determination of their prognostic value. Our experimental research has identified [11–14, 19] that in UC culture cells CTA expression is found with high frequency at early stages. In particular, MAGE – 70%;

ISSN 1562-3629

BAGE – 30%; GAGE – 40%; NY-ESO-1 – 50%. When cultivating, a reduction of CTA quantities expressed by cell lines was noted. CTA expression in samples was non-uniform. When cultivating UC cells for a long time (more than 30 passages), an authentic reduction of percentage of CTA expressing cells – 28.2±4.6% up to their complete disappearance ($p<0.05$) was noted.

Thus, identification of new diagnostic and prognostic markers will allow not only predict the disease itself but determine its run and outcome.

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International Journal on Immunorehabilitation
2018 *Volume 20 No 2*

IMMUNOLOGICAL FEATURES OF HEMORRHAGIC FEVER WITH RENAL SYNDROME

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Hemorrhagic fever with renal syndrome (HFRS) is a feral herd zoonotic infection caused by hantavirus which manifests with hemorrhages, systemic inflammation, and acute kidney injury (AKI). Many HFRS signs can be explained by their immune nature. Predominantly, immunological changes result from the virus. However, they can be also associated with the damage of vascular endotheliocytes and renal cells targeted by the virus. The aim of this study was to address the role of immune cells in the mechanisms of the immune response in HFRS. It was demonstrated that increased cytotoxic T lymphocytes/CTLs ($CD3^+CD8^+$), natural killer T cells ($CD3^+CD56^+$), and regulatory T cells ($CD3^+CD4^+FoxP3^+$ and $CD3^+CD8^+FoxP3$) play the key role in the immune response. In contrast to noninfectious AKI, HFRS is associated with less increase in NK T cells but more significant increase in NKG2D expression by CTLs and NK cells. These findings demonstrate that immunological changes are targeted both to eliminate the virus via cytotoxic lymphocytes and to reduce immune mechanisms of cellular damage in HFRS with active involvement of regulatory T cells.

Key words: *hemorrhagic fever with renal syndrome, acute kidney failure, phenotypes of lymphocytes*.

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UDC 616.15-07; 616.61

Causative agents of the hemorrhagic fever with renal syndrome (HFRS) are ss-RNA hantaviruses (Bunyavirales). HFRS is a feral herd zoonotic infection characterized by a fairly complex pathogenesis [1]. This stems from the fact that the key Hantaan target in the human organism are endothelial cells of small vessels. As a result, the disease manifests with a severe hemorrhagic syndrome, systemic inflammation, and acute kidney injury (AKI) [5].

Most features of HFRS can be explained by pathogenic characteristics of hantavirus as well as by the nature of the immune response to the infectious disease. Many authors suggest that $CD8^+$ cytotoxic T lymphocytes (CTLs) play a key role in the immune responses in HFRS. These cells promote elimination of the pathogen and provide cytolysis of virus-infected endothelial cells thus increasing vascular permeability, hemorrhages, and glomerular injury [5]. Available data also suggest a role for $FoxP3^+$ regulating T cells which suppress immune response and prevent overactivation of $CD8^+$ T cells [7]. Much less information is available on the pathogenic role of innate lymphocytes, in particular, natural killer cells [3]. There are almost no data on the importance of AKI for HFRS development. However, recent published data suggest the effect of noninfectious kidney injury on the immune status [6].

Considering this, the aim of our study was to evaluate the effect of acute kidney injury on the immune responses mediated by various populations and subpopulations of lymphocytes in HFRS.

Patients and methods

The study enrolled 54 patients with HFRS. Clinical diagnosis was verified by epidemiological data and detection of IgM and IgG antibodies against hantavirus nucleocapsid protein using paired serum samples. Comparison group included 8 patients with noninfectious AKI. Control group included 16 healthy individuals.

Blood samples were studied by flow cytometry. The percentage of $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+CD56^+$, $CD3^+CD25high$, $CD3^+CD4^+FoxP3^+$, $CD3^+CD8^+FoxP3^+$, $CD19^+$, $CD3^+CD8^+NKG2D^+$, $CD16^+CD56^+$, $CD56^+NKG2D^+$ cell phenotypes were calculated.

Results and discussion

The levels of various lymphocyte phenotypes in HFRS group, comparison group, and control group are represented in Table 1 and Fig. 1.

In HFRS, small but significant (1.2-fold) decrease in T cells (including T helpers) was revealed as compared with the control group. At the same time, the percentage of regulatory T cells, $CD3^+CD4^+FoxP3^+$ and $CD3^+CD8^+FoxP3^+$, increased by 3.6 times and 31.2 times, respectively. Fairly significant (3.8-fold) increase in CTLs expressing activating lectin receptor NKG2D and 1.4-fold decrease in natural killer (NK) cells expressing activating lectin receptor NKG2D were observed as well.

ISSN 1562-3629

Table 1

The percentage of various lymphocyte phenotypes in the groups

Cells	*Phenotype*	*Median [min; max] (%)*			p_1 p_2
		HFRS (n=54)	*Noninfectious AKI (n=8)*	*Controls (n=16)*	
T cells	$CD3^+$	68.0 [44.4; 86.0]	76.0 [30. 0; 80.4]	75.0 [62; 87]	0.008* 0.184
T helpers	$CD3^+CD4^+$	36.1 [5.5; 58.7]	32.8 [17.5; 52.0]	41.0 [14; 57]	0.038* 0.119
Cytotoxic T cells	$CD3^+CD8^+$	31.8 [10.4; 78.0]	26.5 [12.5; 60.0]	28.0 [16; 71]	0.173 0.390
Natural killer T cells	$CD3^+CD56^+$	5.4 [2.5; 8.1]	13.0 [10.0; 27.0]	3.4 [2.3; 5]	0.041* 0.044*
Natural killer cells	$CD16^+CD56^+$	16.6 [11.0; 33.8]	10.0 [3.4; 15.0]	12.9 [9.5; 28]	0.123 0.458
B cells	$CD19^+$	12.6 [5.0; 25.0]	11.4 [7.6; 15.2]	10.5 [2.5; 16]	0.159 0.275
Activated T cells	$CD3^+CD25^+$	4.2 [2.1; 9.6]	3.0 [1.4; 4.1]	7.4 [2.6; 7.8]	0.108 0.116
Regulatory T cells	$CD3^+CD4^+FoxP3^+$	10.7 [4.0; 27.0]	10.6 [8.4; 12.8]	3.0 [2.3; 8.1]	0.001* 0.034*
	$CD3^+CD8^+ FoxP3^+$	12.5 [3.5; 26.1]	13.2 [2.3; 24.0]	0.4 [0.1; 4.4]	0.001* 0.004*
Cells expressing NKG2D receptor	$CD3^+CD8^+NKG2D^+$	48.6 [23.4; 72]	36.5 [17.0; 75.0]	12.6 [9.6; 27]	0.001* 0.002*
	$CD16^+CD56^+NKG2D^+$	6.7 [1; 16.3]	9.7 [4.1; 18.0]	9.6 [7.7; 21.6]	0.004* 0.078

Note: n – number of patients in each group; p_1 – probability of differences between HFRS group and control group; p_2 – probability of differences between noninfectious AKI group and control group; *reliability of differences using the Mann–Whitney test ($p<0.05$); AKI – acute kidney injury.

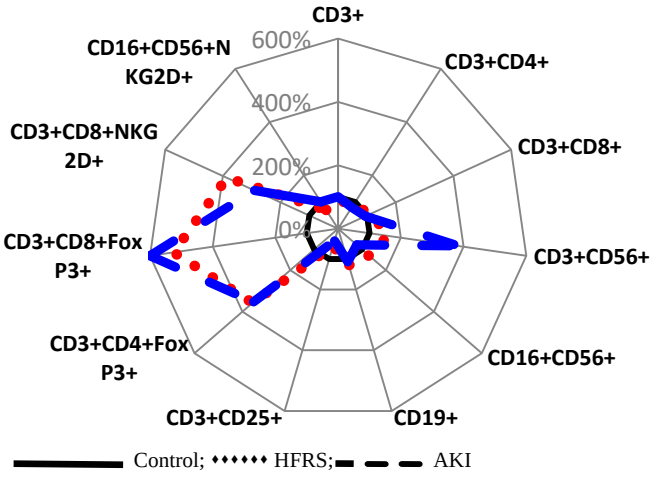

Fig. 1. Deviations (in %) of various lymphocyte phenotypes above the control group in HFRS and noninfectious AKI.

In contrast to HFRS, no changes in the levels of T cells, in particular, T helpers, were revealed in noninfectious AKI. Fundamentally, NK T cell levels significantly (by 3.8 times) increased in noninfectious AKI, i.e., by 2.4 times higher as compared with HFRS. The increase in the levels of regulatory T cells (both $CD4^+$ and $CD8^+$) was almost similar to that in HFRS. NKG2D expression on CTLs increased by 2.9 times in noninfectious AKI and by 3.8 times in HFRS and, therefore, was slightly less in noninfectious AKI. In contrast to HFRS, in noninfectious AKI the levels of $NKG2D^+$ NK cells were similar to those in controls.

Three important issues should be emphasized. First, the role of the cells with cytotoxic activity, i.e., CTLs and NK cells, should be addressed. CTLs are adaptive immune cells

while NK cells are innate immune cells. No significant changes in the percentage of these cells were demonstrated in HFRS or noninfectious AKI. However, significant changes in the expression of NKG2D receptor on these cells were revealed.

In fact, multiple immune cells, i.e., NK cells, NK T cells, γδ T cells, $CD8^+$ T cells, and certain subtypes of $CD4^+$ T cells [4, 9], can express NKG2D receptor. However, membrane NKG2D acts differently depending on the cell type. In particular, signals from this receptor are realized in different ways in NK cells and CTLs. Ligands for these receptors are stress-inducible molecules MICA and MICB on virus-infected and abnormal cell surface [2]. In viral infections, two signal transduction pathways involving adaptor molecules (DAP12 via Syk kinase and DAP10 via PI3 kinase) are initiated in NK cells but signal transduction via DAP10 only is initiated in CTLs [8]. As a result, NKG2D-ligand binding provides cytotoxicity and cytokine release in NK cells. However, in T cells, signaling through the NKG2D leads to their activation only [10].

Our findings demonstrate that NKG2D expression by adaptive immune cells, i.e., CTLs ($CD3^+CD8^+NKG2D^+$), is dramatically increased in HFRS in response to endothelial cell damage by hantavirus. In noninfectious AKI this increase (which is potentially triggered by host damaged cells involving other factors) is much less significant. The lack of the increase or even the decrease in NK cells expressing NKG2D, i.e., $CD16^+CD56^+NKG2D^+$, support this hypothesis. Bearing in mind current theory of the dual (i.e., virus-eliminating and destructive) role of CTLs in HFRS [5], it can be hypothesized that balanced activation of CTL/NK helps to reduce destructive immune effects.

In this light, increased levels of regulatory $CD3^+FoxP3^+$ T cells (both $CD4^+$ and $CD8^+$) in HFRS can be explained as well. Immune system utilizes these cells which suppress cytotoxic reactions to reduce the effects of endothelial and glomerular damage [6]. Our findings on the similar range of regulatory T cell increase in HFRS and noninfectious AKI support this hypothesis.

The role of NK T cells ($CD3^+CD56^+$) which constitutively produce both interferon (IFN) γ, type I cytokine inducing cellular immune responses, and interleukin (IL)-10 and IL-4, type II cytokines inducing humoral immune response [6], should also be highlighted. Type II NK T cells were empirically demonstrated to be the most active cellular subtype in AKI; as a result, hypoxia-induced death of renal tubular epithelial cells is reduced [11]. Our findings suggest that this mechanism (which manifests with a significant NK T cell increase in noninfectious AKI) is significantly reduced in HFRS while protective reactions which prevent cellular damage and improve patient survival are mediated via the mechanisms described above.

Conclusions

In hemorrhagic fever with renal syndrome, immunological changes result from hantavirus and involve T cell (predominantly $CD3^+CD4^+$ T helper) decrease and additional stimulation of NKG2D expression by cytotoxic T cells (but not by natural killer cells). However, immunological changes can also be explained by kidney failure which manifests with acute kidney injury and associated increase in NK T cells ($CD3^+CD56^+$) and, predominantly, regulatory T cells ($CD3^+CD4^+FoxP3^+$ and $CD3^+CD8^+FoxP3$). The pattern of these changes demonstrates that they are potentially targeted to reduce immune mechanisms of cellular damage in HFRS.

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ISSN 1562-3629

NEW APPROACHES FOR ASTHMA MODELLING

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Asthma is a serious global health problem affecting all age groups. Its prevalence is increasing in many countries, especially among children and affecting 1–18% of the population in different countries (GINA 2018). There is no effective treatment for this disease. It is necessary to take into consideration the specificity of pathological processes characteristics of different phases of the disease: acute phase, chronic inflammation phase and remission phase for the developing of adequate methods of treatment. And it is also necessary to create an adequate dynamic model that regards the features of all phases of the disease. For the first time the developed model of asthma, which takes into account the pathophysiological processes of the acute phase of the disease and the phase of chronic inflammation, was applied. The design of experiment includes the approaches of systems biology in order to create networks for asthma for different phases of the disease on the basis of the OVA-induced mouse asthma model. The temporal analysis of the target parameters that were selected on the basis of the methods of system biology were studied in 6 groups of animals, 8 in each group, differing in the time of activation of the receptors of innate immunity: before sensitization, after sensitization and at the stage of provocation. Dynamic competition of humoral (IL-3, 4, 5, 13, IgE, IgA, sCD93) and cellular factors (the number of macrophages, neutrophils, eosinophils, lymphocytes in bronchoalveolar lavage) was first investigated during the airway inflammation when the receptors of innate immunity were activated by fragments of bacterial cell walls, simulating the process of natural immunoregulation, carried out by products of decomposition of commensal microflora. Our findings demonstrate the potential of using of a dynamic model on a base of a multifactor analysis of the mechanisms of exposure of fragments of bacterial cell walls, taking into account the dynamics of humoral and cellular factors in different periods of asthma. This approach can be used to investigate the effectiveness of new medicines for the treatment of asthma and makes it possible to clarify the factors that accompany pathological processes, helping to create an adequate strategy for the treatment of the disease.

AIRBORNE PARTICULATE MATTER INCREASES MUC5AC EXPRESSION BY DOWNREGULATING CLAUDIN-1 EXPRESSION IN HUMAN AIRWAY CELLS

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CLB2.0, a constituent of PM, induces secretion of multiple cytokines and chemokines that regulate airway inflammation. Specifically, IL-6 upregulates CLB2.0-induced MUC5AC and MUC1 expression. Interestingly, of the tight junction proteins examined, claudin-1 expression was inhibited by CLB2.0. While the overexpression of claudin-1 decreased CLB2.0-induced MUC5AC expression, it increased the expression of the anti-inflammatory mucin, MUC1. CLB2.0-induced IL-6 secretion was mediated by ROS. The ROS scavenger N-acetyl-cysteine inhibited CLB2.0-induced IL-6 secretion, thereby decreasing the CLB2.0-induced MUC5AC expression, whereas CLB2.0-induced MUC1 expression increased. CLB2.0 activated the ERK1/2 MAPK via a ROS-dependent pathway. ERK1/2 downregulated the claudin-1 and MUC1 expressions, whereas it dramatically increased CLB2.0-induced MUC5AC expression. These findings suggest that CLB2.0-induced ERK1/2 activation acts as a switch for regulating inflammatory conditions though a ROS-dependent pathway. Our data also suggest that secreted IL-6 regulates CLB2.0-induced MUC5AC and MUC1 expression via ROS-mediated downregulation of claudin-1 expression to maintain mucus homeostasis in the airway.

EFFECTS OF CLIMATIC CHANGES AND AIR POLLUTION ON BRONCHIAL ASTHMA IN WEST GEORGIA

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Epidemiological studies shown that the prevalence of asthma has risen dramatically worldwide and evidence suggests that air pollution factors have an important role in the etiology of the disease.

About climate change it is now widely accepted that the earth's temperature is increasing, as confirmed by warming of the oceans, rising sea levels, glaciers melting, sea ice retreating in the Arctic and diminished snow cover in the Northern Hemisphere. Moreover, changes are also occurring in the amount, intensity, frequency and type of precipitation as well as the increase of extreme weather events, like heat waves, droughts, floods and hurricanes.

However, observational evidence indicates that recent regional changes in climate, particularly temperature increases, have already affected a diverse set of physical and biological systems in many parts of the world.

The aim of the study to establish the correlation between the concentration of phadiatop, total IgE levels in the blood in patients with diagnostic bronchial asthma and the concentration of specific air pollutants in terms of annual calendar of flowering plants in West Georgia.

An individual's response to air pollution depends on the source and components of the pollution, as well as on climatic agents. Indeed, some air pollution-related episodes of asthma exacerbation are due to climatic factors that favour the accumulation of air pollutants at ground level.

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In the study, were involved 65 patients (among them 34 males and 31 females) of different ages, with diagnostic bronchial asthma (according to GINA recommendation) who for allegro-specific diagnostics applied to the S/R Institute of Allergology, Asthma and Clinical Immunology of Georgian Academy of Sciences (Tskaltubo, Georgia) from January to April, 2017. The study included the following stages of allegro-diagnostics: I step – allergodiagnostic using modern automated system – "Immuno CAP 100" (PHADIA, Switzerland); II step – Monitoring of aeropollutants concentration by using aeropolinometer "Burkard Trap" (Great Britain). The analysis of the laboratory results showed that the studied patients had high titers of total IgE, which amounted to an average of 273 (N 33–90), while the average concentration of phadiatop was 96 (N<70), respectively. In the patients with bronchial asthma of a specific positivity of specific IgE to the weeds (Wx2) – ambrosia, plantain, clasp/tarragon, atriplex – in 25 (55%) on average; tree dust (Tx9) – alder, lactarius piperatus, nuts, oak, willow – 16 (35%); and cereals (Gx1) – *Festuca pratensis, Lolium temulentum, Timoti grass*, poa – 8 (17%); Mx2 – *Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Candida albicans, Alternaria alternate* – 11 (24%) was revealed, only in 6 (13%) patients we cannot established the allergy specific IgE. From January to April 2017, there were revealed a high concentration of aeropollutants, by high allergenization and widespread; especially high concentrations were found in alder, birch tree and common hazel, while from aeropollutants of low allergenization poplar, elm, willow and plane tree were distinguished. A concentration of different types of tree-dust and surrounded atmospheric aeropollutants was specified by using aeropolinometer "Burkard Trap" at a given period of time and consequently, the annual calendar for distribution of aeroallergens in West Georgia was developed over again. High degree correlation between the above-mentioned markers proves its clinical importance/value with respect to bronchial asthma.

NEW ASPECTS IN THE CLINICAL-IMMUNOLOGICAL APPROACH OF PATIENTS WITH ATOPIC DERMATITIS

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Introduction: Scientific studies devoted to the particularities of the immune response in various evolutionary forms of atopic dermatitis (AD) in patients of different age denote controversial character. Humoral immune factors and cytokines play an obvious role in AD evolution. The testing of humoral indexes in AD patients would make it possible to identify specific particular lesional forms that would facilitate the treatment with immunotropic remedies.

Material and Methods: The study included 110 AD patients and 110 apparently healthy children who were assigned to two age groups (group I – up to two years and group II – 2 to 18 years). Immunological testing of blood serum included assessment of IgG, IgG, IgA, IgE – total) immunoglobulin concentration as well as cytokines (IL-2, IL-4, TNFα) depending on the severity of the disease (SCORAD) of lesional forms and association with IgE.

Results: The analysis of the obtained materials demonstrated a decrease of the IgA level with the increase of the total IgE concentration and the cytokines studied in the blood serum of the AD patients compared to the respective control groups. These changes were more pronounced in group II patients in severe and moderate forms. There was a more significant increase in TNFα cytokines and less expressed IL-2 in exudative form in both groups. There was a significant increase in IL-4 in the lichenified form of group II. Evolutionary growth trend of TNFα was recorded in exudative form in children of group II and decreased in subjects up to 2 years of age. There was a decrease in IgA concentration in both groups, more significant in the intrinsic form. We also established a positive correlations between IgE total and IL-4 in eritemato-scuamous and eritemato-scuamous lichenified forms. Total IgE dynamics was net ascending in extrinsic form.

Conclusions: A biphasic evolution of the immune response in patients with AD has been established, which in incipient (under 2 years), erythemato-squamous and exudative, extrinsic forms are accompanied by increases in total IgE and IL-4. After 2 years of age, patients with lichenified, intrinsic forms, demonstrated an immune response with increased TNFα and IL-2.

ANALYSIS OF ASSOCIATIONS OF THE CONTROL FACTOR AND CLINICAL AND GENETIC PARAMETERS IN CHILDREN WITH BRONCHIAL ASTHMA

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The urgency of the problem. Control of bronchial asthma (BA) should be considered as the main goal of therapy of this disease and, at the same time, as the most important marker of the quality of medical care. For the last period, it remains relevant to search for new methods for a more simple and accurate assessment of the level of control over asthma. The most effective of them are considered questionnaires, which allow to reflect the control over the disease. Materials and methods: a cohort of patients was studied in the number of 177 children, both sexes, diagnosed with bronchial asthma, the severity of the disease was different. Analysis of control factor associations was carried out using conjugacy tables. The level of control was set in two different ways: three-level (no control, partial control, controlled by the BA) and two-level (0 – no control + – partial control, 1 – controlled BA). The following indicators were taken as dependent variables: sex, age, severity of disease, debut heredity, length of illness, FEV_1, total Jg E, presence of allergic rhinitis. From the genetic parameters, the genes of cytokines TNF (G308A), IL-4Rα (Ile50Val), IL-4, (C-590T), ADRβ2 (Arg16Gly) plasma endothelin-1, autoantibodies to collagen 1 and call type 3, fibroblast growth factor basic form). A logistical model was also constructed separately

ISSN 1562-3629

on genetic factors. Results and conclusions: in the analysis of clinical-anamnestic and genetic parameters, statistically significant results were obtained. It was established that AR is a predictor of ineffective control of asthma ($\chi2=5.5$, $p<0.01$), an association of ineffective control and polymorphism by ADRβ2 (Arg16Gly) ($\chi2=5.6$, $p=0.05$). The logistic model on genetic parameters had a low specificity (69.57%) and sensitivity (66.67%).

TOBACCO SMOKE EXPOSURE IN CHILDREN POPULATION WITH ALLERGIC RHINITIS, BRONCHIAL ASTHMA AND ATOPIC DERMATITIS

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Respiratory system diseases are considered as a global issue throughout the world. The aim of the study: investigation of tobacco smoke exposure and quantitative and qualitative evaluation of developmental risk factors in school age children population of Tbilisi and Kutaisi affected with allergic rhinitis, bronchial asthma and atopic dermatitis.

Materials and Methods: the study was conducted according to the survey carried out in random and representative contingent of school age children by using cross-sectional method of one-moment epidemiological research. Schools and study groups were selected randomly. The first phase of the study was conducted in 2350 (boys – 59.2% and girls – 40.8%) children, 13 to 18 years of age.

Results: Allergic rhinitis prevailed in patients with: atopic dermatitis (21%), bronchial asthma (36.2%) compared with healthy ones (12%), the difference was statistically reliable ($p<0.01$). Family history of allergic diseases was also frequently observed in patients with bronchial asthma (29.2%/2%, $p<0.01$). Tobacco smoke exposure was revealed in 72% of children Population with bronchial asthma, while tobacco smoke exposure was observed in 67% of patients with allergic rhinitis, atopic dermatitis prevailed in patients (75.9%) with allergic rhinitis and bronchial asthma in comparison with healthy ones (69%), difference was statistically reliable ($p<0.01$) in comparison with healthy children –only 22% ($p<0.05$).

Conclusion: based on the study results it can be concluded that the tobacco smoke exposure is a significant risk factor for development of allergic rhinitis, bronchial asthma and atopic dermatitis. According to the obtained data, the specific gravity of the manageable risk factors that might be the basis for development of targeted and effective measures to prevent allergic rhinitis, bronchial asthma and atopic dermatitis in children population is very high.

THE ROLE OF IL17 IN THE DEVELOPMENT OF FOOD HYPERSENSITIVITY AND METABOLIC DISTURBANCES

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IgG indices of mediated food antigens hypersensitivity (fAG) are an accessible criterion for changes in food tolerance (FT) associated with quantitative characteristics of food, the quality of their digestion, and the state of the immune system of the intestine. Immunoregulation FT provided a combination of factors: genetics digestive enzymes, functional state microbiota TLR expression on enterocytes, sIgA products, IgG, TGFβ, IL-10 activity of Treg, Th17 cells. In this regard, attention should be paid to IL-17, which is associated with maintaining the immune homeostasis of the intestinal mucosa. ***Purpose:*** To evaluate the contribution of IL17 to the development of IgG responses to mediated food hypersensitivity and metabolic disorders in patients with increased body weight.

Materials and Methods: Venous blood and serum of volunteers with different body mass index (BMI). 70 volunteers 27 3) OR=2.3 (2.8, 23.9), and increased IL-17 concentration was statistically significant ($p<0.05$), a statistical relationship was established between IL-17 and C-reactive protein ($p<0.05$). We assume that IL-17 participates in the development of food hypersensitivity to milk proteins, soy, gluten and metabolic disorders in patients with elevated BMI.

IMMUNOLOGIC SIGNS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME

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Hemorrhagic fever with renal syndrome (HFRS) – infection caused by hantavirus and manifested itself in the form of the haemorrhagic syndrome, the systemic inflammation and acute kidney injury. This infection has wild-localized zoonotic character. At the heart of many manifestations of HFRS lies their immunological nature. Immunological developments largely were due to a virus but may be related to damage of vascular endothelial cells and kidneys as targets for viral pathogen. The purpose of this study was the definition of the role of immune system cells in the mechanisms of development of an immune response in HFRS. To achieve this goal, the blood of 54 patients with HFRS, 8 patients with noninfectious acute kidney injury, 16 healthy people have been investigated using the method of flow cytometry. It was found that the key value in the immune response in HFRS growth of cytotoxic T cells ($CD3^+CD8^+$), NKT ($CD3^+CD56^+$), regulatory T cells ($CD3^+CD4^+FoxP3^+$ and $CD3^+CD8^+FoxP3$) in the blood plays. Unlike noninfectious acute kidney injury HFRS is associated

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with more moderate growth of the number of NKT, the more pronounced the expression level of activating lectin receptor NKG2D by cytotoxic T lymphocytes as well as by natural killer cells. Observed changes show their possible focus not only on elimination of viral pathogen with the participation of lymphocytes with cytotoxic properties, but also to limit the immune mechanisms of damage of target cells in HFRS with active involvement of regulatory T cells in the immune process.

RISK FACTORS FOR FALLING SENSITIVITY TO ANTIVIRAL THERAPY IN CHRONIC HEPATITIS "C" PATIENTS

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Chronic hepatitis C (CHC) is now moved into the category of controlled diseases. This is related to the introduction of antivirals of direct anti-HCV activity in the clinical practice, however, the availability of these products on the Russian market is often limited by economic considerations. In this regard, prediction of sensitivity of patients to different schemas antiviral therapy remains relevant, and the identification of risk factors of falling such sensitivity was the objective of the present study. 437 patients with CHC were observed, 242 of them received pegylated interferon α and Ribavirin, in 41 patients this therapy was supplemented by direct-acting drug ("triple" therapy) 154 men received only direct-acting medications. Risk factors of falling sensitivity to the drugs mentioned were conducted differently in patients receiving antiviral therapy for the first time, in previously and unsuccessfully treated patients, in patients with established cirrhosis of the liver, as well as in the presence of concomitant liver steatosis or chronic alcoholism. The identified risk factors included demographic data of patients, hepatitis C virus genotypes, polymorphisms of genes of IL-28B, adiponurin, inducible nitric oxide synthase, activating lectin receptor NKG2D in various polynucleotide sequences. It was found that most frequently falling sensitivity was observed during therapy with pegylated interferon α and Ribavirin (38–48%) and the largest number of risk factors were found in groups with cirrhosis and concomitant chronic alcoholism. "Triple" therapy sensitivity drop does not exceed 25% and had a genetic basis, when treating drugs direct action noted only in 1 person out of 154.

UROTHELIAL CANCER: NEW TRENDS IN DIAGNOSTICS AND PROGNOSTICATION OF THE CLINICAL OUTCOME

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Urothelial cancer (UC) is one of the leading pathologies among urological cancers and covers 13% in the total mortality structure. It's extremely scanty manifestations at early stages of the disease lead to its late identification. An insufficient informative value and limited abilities of currently used diagnostic methods have been noted, due to the fact their improvement, as well as development of new methods and markers of early diagnostics and prognostication of the disease with maximum specificity, receptiveness and informative value are ongoing. Basic researches carried out during the last decade were focused on the optimization of UC histological and genetic criteria. It has been established that molecular genetic disorders play a significant role in the development of cancers, and their investigation allows outlining new trends in diagnostics and prognostication of the disease.

We have carried out a comprehensive analysis of modern national and foreign literature based on which the UC phenotype research data and the molecular pan-cancer analysis results have been presented; new UC classification data based on the research of the genetic profile of various UC forms have been presented; we have made a brief review of the researched molecular genetic markers of early genomic alterations related to UC molecular variety. Currently two separate networks of genomic alterations related to UC molecular variety which can reflect various ways of tumor growth: surface (muscle-non-invasive) and muscle-invasive, have been identified. 5 molecular subtypes of UC have been determined: Urobasal A (UroA), Urobasal B (UroB), GU, SCCL and the infiltrated subtype characterized by predominance of expression of non-tumour phlogistic transcriptases. Today, a DNA methylation analysis; genetic networks of lipid metabolism regulation; RNA role; FOXM1 and PLK1, FOXM1, PPARG, FOXA1 and GATA3 protein expression levels; basal keratin expression, etc. are actively researched. It has been established that FGFR3 and PIK3CA (a cathetic subunit of PI3-kinasa α) mutations play a key role for muscle-invasive tumors; it has been noted that in approximately 20% cases activation of genes of the RAS (HRAS1, KRAS and NRAS) family can be seen and takes place as a result of point mutations in 12, 13 and 61 codons of these genes. *In situ* cancers are characterized by mutation of tumor growth suppressor genes – TP53, RB and PTEN. Among diagnostic markers proposed today only six of them have been approved and are used in Europe and America for early identification of UC: BTAStat, BTA TRACK, NMP-22, BladderChek, ImmunoCyt and UroVision. Research of cancer/testis antigens: NY-ESO-1, MAGE-A3, LAGE-1 and PRAME holds much promise for identification of their prognostic value. Our experimental investigations have identified that UC cell cultures at early passages displayed the CTA expression with a high frequency namely; they are MAGE – 70%; BAGE – 30%; GAGE – 40%; NY-ESO-1 – 50%. While cultivating, a reduction of CTA quantities expressed by cell lines has been determined. The CTA expression in the samples was not uniform. When cultivating UC cells for a long time (more than 30 passages), an authentic reduction of percentage of cells expressing CTA – 28.2±4.6%, up to their complete disappearance ($p<0.05$), has been identified.

ISSN 1562-3629

INNOVATION TECHNOLOGIES IN TREATMENT OF UROTHELIAL CANCER

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Urothelial cancer (UC) ranks nine among cancers around the world. Its early stages are featured by lack of clinical manifestations that doesn't allow assigning an adequate therapy. Treatment (T) of invasive metastatic cancer involves considerable difficulties. UC T at this stage includes as follows: surgical T (transurethral resection with or without photodynamics (FD) and cystectomy depending on cancer invasion degree), chemotherapy – ChT(systemic or local), radiation T (RT) and immunotherapy (IT). We have developed the new modified photodynamic (FD) complex T method (CT) for superficial surface urothelial cancer. In this case we have noted 5-years relapse free progress of urothelial cancer in 85.2% cases in patients with single tumors and in 53.8% cases of multiple UC lesions. Patients with single and multiple tumors who received CT showed 80% non-relapsive cases. Such cases in patients treated conventionally (TC) were 45%. Specific survival of UC cases with CT was 98%, as compared to 92% cases with CT ($p \leq 0{,}005$). IT of UC plays a significant role. The non-specific UC IT comprises of: intravesical BCG-IT; cytokine therapy – CyT (independently or combined with ChT and radiation T) and adoptive IT (*in vitro* activated immune cells infiltration into the body). Modern UC T regimens provide recombinant IL-2 use or its combination with effector cells such as LAK and TIL, peptide vaccines; use of CyT/immunoChT. The specific UR IT includes: targeted therapy, blockade of immune checkpoints, vaccine therapy. The targeted therapy using monoclonal antibodies is a breakthrough in the T of cancer patients. For such therapy used such preparations for which the growth factors and the UC cells expressed receptors are the targets. Blockade of immune checkpoints is used to block the check system and to recover the antitumor immune response. Such T is provided by preparations such as anti-CTLA-4 (Ipilimumab), anti-PD-1 (Pembrolizumab and Nivolumab) and antiPD-L1 (Avelumab, Atezolizumab) that showed high treatment efficacy of metastatic UC. Prospective IT methods include peptide, idiotypic, allogenic or autologous DNA vaccines. Autologous dendritic cell (DC) vaccines can initiate and potentiate tumor antigen-specific reactions by activating T-cell component of immune system. We undertook some experimental studies to develop the autologous DC-vaccine for UC T during which the conditions for tumor cell culture were established as well as cells selection criteria were determined to develop such vaccine. Gene therapy is the new cancer treatment method, but there are no similar studies focused on UC T.

Thus, modern molecular genetic studies and achievements in the field of fundamental immunology allowed not only develop the new innovative UC T technologies but also prove molecular genetic relation with different clinical forms of the disease.

IMMUNOGENETIC RISK CRITERIA FOR CERVICAL CANCER

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Relevance: Oncological diseases occupy the leading positions in the structure of mortality in patients of working age. Studies (IBSCC) have shown that HPV 16 and 18 types are presented in 99.7% of samples. To date the dominant theory that explains the occurrence of cervical cancer is viral-induced. Moreover, the mechanism of the occurrence of cervical cancer in viral contamination in women has been described in the literature extremely contradictory and there is no connection between the genetic markers of cervical cancer and the individual components of the immune system.

Goal: To develop immunogenetic criteria for the risk of cervical cancer.

Materials and Methods: The study involved 120 women with voluntary medical consent aged 19 to 42 years. Distribution by group, taking into account the classification of R.M. Richar (1968) and TBS (2001 revision) was as follows: 1 gr. – 26 healthy women (HPV 16, 18 types – 2 gr. (LSIL) – 52 women with CIN I–II st., Dysplasia (HPV16/18 types+), 3 gr. – 31 women with CIN III degree (HSIL) and 4 gr. – 11 women with cervical cancer *in situ* (HPV16 18 types+). The study of cells with the phenotype $CD3\text{-}CD16^{+}CD56^{+}$ was carried out in biopsies of the cervix uteri.

Results: In the 1 st gr. the level of $CD3^{-}CD16^{+}CD56^{+}$ cells was 6.82±1.45% and correlated with histocompatibility antigens of class 2 HLADR1. In the 2 nd gr. (HPV16/18+), the number of $CD3^{-}CD16^{+}CD56^{+}$ was 13.7±1.009%, a comparison in 86.3% of cases with the HLADR1 genotype. In the third group. (HPV16/18+), the mean $CD3^{-}CD16^{+}CD56^{+}$ cell count was 18.6±1.12%. The genotype was presented by the reduced potassium activity of HLAB35 (14.2%) and HLADR2 (12.6%). In women with cancer *in situ*, the level of $CD3^{-}CD16^{+}CD56^{+}$ cells was 11.4±0.92%, and the genotype was represented by HLAB35 (63.6%).

Conclusions: The number of natural killers associated with genetic indicators is a criterion of an infectious-induced process, including non-communicable one.

$CD39^{+}FOXP3^{+}$ REGULATORY T-CELLS IN COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the most common form of malignant tumors in Russia and in the world. The pathogenesis of CRC is followed by changes in cellular immunity, in particular subpopulations of regulatory T-cells (Treg). The

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aim of this study was to assess the activity of FOXP3$^+$ Treg-cell subpopulation that express CD39 surface marker (ectonucleotidase ENTPD1). Peripheral blood samples of 30 CRC patients (mean age 63.0±17.9 years) and 20 healthy donors (55.2±18.1 years) were investigated. The diagnosis of CRC and disease stage was established according to TNM classification. Sampling and the analysis of lymphocytes in CRC cohort was carried out prior to therapy. The numbers of Treg-cells were estimated by multi-parameter flow cytometry on the FC500 (Beckman Coulter, USA) with application of antibodies to membrane and intracellular markers: CD4, CD25, CD127, FOXP3 and CD39 (Beckman Coulter, USA; eBioscience, USA). Researches were carried out on the equipment of the KarRC RAS core facility. The differences between groups were estimated by Mann–Whitney's criterion ($p<0.05$). We have established significant increase in CD39$^+$ Treg-cell percentages as compared to control. The level of expression of ectonucleotidaze CD39 was significantly higher on FOXP3$^+$ Treg-cells of CRC patients (57.9±6.8% against 43.9±5.4% in control cohort). Also we found significant changes in CD39$^+$ cell numbers at different stages of CRC. At later stages of CRC (III, IV) the CD39$^+$ cell level was higher, than during earlier stages (I, II) (68.3±7.6% and 41.9±9.3%, respectively). During oncogenesis CD39 takes part in regulation of immune suppressive mechanisms. In this work we demonstrate the increase in CD39$^+$ of Treg-cell numbers in CRC patients, which may reflect the up-regulation of functional activity of these cells. *The reported study was supported by Russian Science Foundation (Project № 17-75-10182).*

THE INCIDENCE OF MALIGNANT BONE TUMORS IN THE REPUBLIC OF AZERBAIJAN IN 2017

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The purpose of this study is to study some aspects of the incidence of malignant bone malignancies in the Republic of Azerbaijan in 2017. For this purpose, we analyzed the incidence of malignant bone tumors with the calculation of medical and statistical indicators used in oncology. In the structure of the incidence of malignant neoplasms, this nosology is 1.5% (for males – 1.8%, and for females – 1.2%). The incidence rate is relatively low and is based on the intensity of the incidence rate of 1.70/0000 (for men – 2.00/0000, for women –1.40/0000). The calculation of the standardized morbidity rate revealed that male subjects are 1.8 times more likely to fall ill than females (2.00/0000 *vs.* 1.10/0000, respectively).

DIFFERENT KINDS OF INTERFERON-CORRECTIVE THERAPY IN CONGENITAL AND ACQUIRED INTERFERONOPATHIES

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Interferons (IFNs) play a key role in immune defense against viral and bacterial infections. At first, in case of contact with nucleic acids (RNA, DNA) of viruses, the synthesis and the secretion of IFNs are activated. Then IFNs type I (IFNα and IFNβ) and IFN type II (IFNγ) implement their effects via cognitive receptor complexes IFNAR and IFNGR that are expressing different immune and non-immune cells. Few years ago, type I interferonopathies associated with overexpression of interferon alfa was described in rare Mendelian genetic diseases, certain autoimmune diseases, immune dysregulation syndrome. To-day we understand that different kind of interferonopathies exist. Interferonopathies are congenital and acquired. Based on our own experience, on the experience of Russian and foreign researchers, we have developed the interferonopathies classification**:**

I. Congenital interferonopathies: 1. Interferon deficiency**:** 1.1 Interferon α deficiency (IFNα) and IFNAR deficiency); 1.2. Interferon β deficiency (IFNβ); 1.3. Interferon γ deficiency (IFNγ) and IFNGR deficiency); 2. Interferon overexpression**:** 2.1. IFNα overexpression: 2.1.1. Autoinflammatory syndromes and autoimmune diseases (systemic lupus erythematosus, systemic angiitis, dermatomyositis), Down syndrome; 2.1.2. Type I interferonopathies: Aicardi–Goutières syndrome, familial chilblain lupus, spondyenchondromatosis, proteasome-associated auto-inflammatory syndrome (PRAAS), Singleton–Merten syndrome.

II. Acquired – secondary interferonopathies: 2.1. IFN deficiency: 2.1.1. Interferon α deficiency (IFNα); 2.1.2. Interferon β deficiency (IFNβ); 2.1.3. Interferon γ deficiency (IFNγ); 2.2. Interferon system paralysis: 2.2.1. Adequate IFNα response block; 2.2.2. Adequate IFNβ response block; 2.2.3. Adequate IFNγ response block;

III. IFNs overexpression Different kinds of the interferonopathies need in different interferon-corrective therapy methods. The targeted type I interferonopathies therapy is aimed at blocking IFNα overexpression. Most frequent interferonopathies by IFN deficiency type meet: congenital or acquired IFNα/β and IFNγ deficiencies in children and adults, associated with abnormal viral or mycobacterial infections. Replacement IFNα-therapy is indicated for patients with congenital deficiency of IFNα. In case of acquired IFNα deficiency the differentiated interferon-corrective therapy is performed. In both replacement and interferon-corrective therapies recombinant human IFNα2b in combination with antioxidants – viferon is used, which is safe and has good clinical efficiency and no side effects.

 ISSN 1562-3629

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2018 *Volume 20 No 2*

XI WORLD ASTHMA, ALLERGY & COPD FORUM
♦
XXV WORLD CONGRESS ON REHABILITATION IN MEDICINE & IMMUNOREHABILITATION
Barcelona, Spain ***April 20–23, 2018***

EXPERIMENTAL REMODELING IN VITRO OF TRANSFORMED CD16⁺CD32⁺CD11B⁺ PHENOTYPE SUBSET OF NEUTROPHILIC GRANULOCYTES IN PATIENTS WITH ATYPICAL CHRONIC BACTERIAL INFECTION

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Background: Today it has been shown that there are subpopulations of neutrophilic granulocytes (NG), which have both activating, modulating, and suppressive regulatory influences. They have plasticity – a dynamic change in phenotype and properties under influence of endo- and exogenous origin factors, thus NG determine the course of the infection inflammatory process. The aim of the study was to investigate the possibility of remodeling the transformed phenotype $CD16^+CD32^+CD11b^+$NG subpopulation under the influence of regulatory peptide-glucosaminylmuramyl dipeptide (GMDP) and regulatory cytokine IFNγ *in vitro* in patients with secondary immunodeficiency associated with atypical chronic sinusitis (CS) recurrent course.

Materials and Methods: In study were included 10 patients of both sexes, 38–60 years old, with CS in acute phase. Control group consisted of 10 conditionally healthy volunteers comparable by sex and age. In study were performed: NG phenotype in intact blood and after influence of GMDP and IFNγ *in vitro*, % CD16CD32CD11b positive NGs and mean fluorescence index (MFI).

Results: It was found that in the peripheral blood of healthy individuals and patients with CS 86,7–96,7% NG presented a subset of $CD16^+CD32^+CD11b^+$, with different levels of expression of these receptors. In patients with CS was detected NG subset CD16dimCD32midCD11bbr, in control group – CD16brCD32brCD11bdim. The analysis of the results allowed revealing violations of NG functional activity – lack of activation and adequate response in case of chronic bacterial process exacerbation. In the study significant increase in the expression of receptors providing NG effector properties under the influence of GMDP was established. Also we noted the appearance of CD16brCD32midCD11bmidNG in patients with CS and CD16brCD32brCD11bbrNG in control group. The effect of IFNγ was the appearance of CD16dimCD32dimCD11bdimNG in patients with CS, which is probably illustrated inclusion of regulatory mechanisms aimed at the regressing of inflammatory response in exacerbation of CS.

Conclusion: The studies demonstrate presence of transformed CD16dimCD32midCD11bbr NG phenotype in patients with secondary immunodeficiency associated with atypical persistent recurrent course CS.

PHENOTYPIC CHANGES OF IFNαβR1⁺CD119⁺CD284⁺ NEUTROPHILIC GRANULOCYTES IN CHRONIC HERPES-VIRAL INFECTIONS AND THEIR POSITIVE TRANSFORMATION BY RECOMBINANT IFNα2 IN THE "SYSTEM *IN VITRO*"

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Neutrophilic granulocytes (NG) are multipotent cells of the immune system, which participated in anti-viral protection. It is shown that in cases of chronic herpes-viral infections (ChHVI), neutropenia, disturbances in the interferon system (IFN) are often observed. In this connection, it is of interest to study the phenotypic features of IFNαβR1⁺CD119⁺CD284⁺NG in ChHVI and to develop methods for their correction. Under our supervision there were 18 people aged between 23 and 60 years, both sexes, suffering from ChHVI. The presence of various mono- and mixed ChHVI in the stage of viral replication was confirmed by the methods of sero- and PCR diagnostics. The control group consisted of 10 people, comparable by sex and age. Methods of flow cytofluorimetry were used to assess the phenotypic features of NG. An adequate statogram with Me[Q1; Q3], Mann–Whitney test (MWU-test), 95% CI-reference control values was carried out. In the control group, the amount of IFNαβR1⁺NG (%) was 2.50 [1.25; 3.55], the expression density of IFNαβR1⁺ was 1.09[1.07; 1.34]. 95% CI was 1.14–3.79. The amount of IFNαβR1⁺NG in patients with ChHVI were different. In group 1, IFNαβR1⁺NG (%) was 3.05 [2.70; 3.75] and did not differ from the control level (MWU-test=0.26), in group 2 IFNαβR⁺NG(%) was 5.35 [4.93; 8.03], which significantly differed from the control (MWU-test=0.001). At the same time, according to MFI, there were no differences from the control in both groups. In the control, the amount of CD119⁺NG (%) was 19.60 [14.85; 28.60], MFI-1.15 [1.12; 1.19]. In persons suffering from ChHVI, the number of CD119⁺NG (%) was 28.95 [22.15; 38.00], which significantly differed from the control (MWU-test=0.02). According to MFI, there were no differences from the control (MWU-test=0.162). The number of NG (%) bearing TLR4 was 8.55 [6.40; 10.23], with the MFI level – 1.14 [1.07; 1.16]. In patients of group 3 with ChHVI, the number of NG(%) bearing TLR4 was 12.80 [9.70; 16.65] , which differed from the control level (MWU-test =0.03) and 6.80 [6.05; 7.00] in the group 4 (KMU =0.06). At the same time, the MFI level did not differ significantly from the control. Incubation of NG patients in the "*in vitro* system" with the rec. IFNα2 (at the final concentration of 50 μM/μl) significantly decreased the amount of IFNαβR1⁺NG (%) to 2.40 [1.33; 3.23], both in the first and in the second group MWU-test 1=0.001, MWU-test 2=0.0001), which entered the 1.5-σ control zone. At the same time, the density of receptor expression (MFI) increased (p=0.0001). Apparently, the decrease in the amount of IFNαβR1⁺NG (%) appeared against the binding of IFNα2 to IFNαβR1. Under the influence of the rec. IFNα2, the number of CD119⁺NG (%) significantly decreased, to 15.50 [12.55; 24.80] (MWU-test=0.007) and entered the 1.5-σ control zone. At the same time, the density of receptor expression (MFI) changed insignificantly (MWU-test=0.02). Rec.IFN-α2 significantly reduced the number of NG (%) bearing TLR4, to 4.40[3.00; 8,10], which is below the 1.5-σ control zone. The density of expressing TLR4 (MFI) did not change. Thus, various transformations of the phenotype of the subpopulation IFNαβR⁺CD119⁺CD284⁺NG have been identified. Rec. IFNα2 in the "*in vitr*o system" corrects the altered phenotype of the subpopulation IFNαβR1⁺CD119⁺CD284⁺NG.

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STUDY ON THE ETIOLOGICAL STRUCTURE OF MORBIDITY AND FUNCTIONING FEATURES OF THE ANTIVIRAL PROTECTION SYSTEM IN PATIENTS WITH HERPESVIRUS INFECTIONS

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In recent years, herpetic infections have occupied a special position in the infectious diseases structure. These infections are characterized by a high prevalence, high virus carrying frequency, atypical course and a diverse clinical picture, as well as the complexities of clinical and laboratory diagnostics. The herpetic infections occur in the form of mono, mixed and co-infections and can be asymptomatic (latent), in acute, chronic persistent form with recurrent course, and also in the form of atypical chronic active infection (ACA).

Objective: to study the etiological structure of the morbidity of mono- and mixed herpes virus infections in adults; to evaluate the functioning features of the antiviral protection system in this group of patients.

Materials and Methods: We have observed 198 patients aged between 23 and 60 years old, suffering from mono and mixed herpes virus infection. In addition to the traditional methods (collection of anamnesis, methods of physical examination, CBC, etc.) for the detection of herpesviral infections, serological tests were used (IgM VCA EBV, IgG VCA EBV, IgM CMV, IgG CMV IgM HSV1/2, IgG HSV1/2) using the ELISA test systems of the "Diagnostic Systems" SPA (Russia), as well as the PCR test system "AmpliSens" (Russia) to detect the genome of viruses in biomaterials (blood, saliva, urine, scraping from the tonsils and the posterior pharyngeal wall). To evaluate the features of the functioning of antiviral immunity (immunogram, INF-status, etc.), flow cytometry and ELISA methods were used.

Results: According to the data obtained, 36.6% of patients suffer from mono-infections, 55.5% of them are patients with EBV infection; 35.3% with HSV type 1 and 11.1% with HSV type 2 and CMV, respectively. 63.7%. of patients were infected with herpetic mixed infections. In the structure of these infections, combinations of $HSV1^{+}$HSV type 2 are leading; EBV^{+}HHV type 6, $EBV^{+}CMV^{+}$HHV type 6, and also EBV CMV^{+}HHV type 6^{+} HSV type 1 – of 11.2%. A further distribution of mixed infections according to the combinations occurrence is as follows: EBV^{+}CMV (9.7%); $EBV^{+}CMV^{+}$HSV 1 (4.8%); $EBV^{+}CMV^{+}$HSV 2 (3.2%). The most pronounced disturbances in the IFN system were found in herpetic mixed infection. Reduction in induced production of IFN-α and IFN-γ was present in 100% of patients and was more pronounced than in mono- infection. Infringements and an imbalance in a population structure of blood lymphocytes were revealed: deficit $CD3^{+}CD8^{+}$ cells – 70%, NK – 80%; EKT– 67%. In CBC – neutropenia and lymphocytosis in 100% of cases.

Conclusion: During the research, the etiological structure of mono- and mixed herpes virus infections in adults was studied, the main functioning features of the antiviral immunity system were revealed.

IMMUNOLOGICAL PROFILES OF GESTATIONAL DISORDERS

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Relevance: The dominant role in the processes of gestation is given to the immune system. Its condition determines the success in achieving the ultimate goal – the birth of a healthy full-term child.

Purpose of the Study: The study of immunological parameters in women with gestational disorders.

Materials and Methods: The design of the study was presented by 171 pregnant women. Physiological course of pregnancy was registered in 60 women, 57 women – threat of interruption, 54 women – moderate forms of gestosis. Cellular studies were performed using monoclonal antibodies in accordance with the nomenclature of antigens. The cytokines were determined using reagent sets "IL-1, 2, 4, 6, 8-ELISA-BEST", "alpha-TNF-ELISA-BEST", "alpha, gamma-INF-ELISA-BEST". Functional parameters were evaluated in RTML with PHA and placental antigens (intrinsic modification).

Results: Immunotype in the physiological course of pregnancy fits into the physiological variant of immunosuppression. RTML with PHA within normal limits. Diametrically opposite values were obtained in women with the threat of termination of pregnancy. The most pronounced changes affected cells with markers CD25 and CD16. Immunotype of women with gestosis was characterized by pronounced suppressor changes. In the study, significant changes were observed in cells with markers CD3, CD4, CD8. As a result of subpopulation changes, IRI decreased to 1.1±0.106. At women with gestosis marked abrupt changes in functional fullness by the type of cellular incompetence. Thus, the conducted researches allow to recommend in practical public health services detailed researches of cellular-cytokine parameters at various types of gestation for more exact diagnostics of gestational complications.

SYNTHICIAL CONNECTIONS OF NEUROICITIS IN VEGETATIVE GANGLIAS OF THE INTESTINE

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Introduction: The theory of the reticular nerve network, as a principle of the organization of the nervous system, formulated by G.I. Fon Gerlach in 1879 was supported by most neurologists of the 19th century, including Camillo Golgi. However, since the reticular theory did not have scientific facts obtained at the light-optical level, it was replaced by the neural doctrine of Santiago Ramon-i-Cajal (Sotnikov O.S., 2013). For the first time A.S. Dogel (1893) succeeded in presenting real

ISSN 1562-3629

drugs that indicate the presence of commissural syncytial connection between the bodies of neurocytes. Even earlier in 1877, A.I. Babukhin discovered the possibility of conducting a nerve impulse in opposite directions, refuting the most important law of the neural theory "on the dynamic popularization of the nerve impulse."

The aim of the work is to present morphological evidence of the presence of syncytial connections of neurocytes in vegetative ganglia of the intestine of mammals.

Materials and Methods: Preparations of the submucosal and intermuscular nerve plexuses of the intestine of sexually mature outbred rats (n=15) were studied. The thickness of the preparations is 170.0-–220.0 μm, the area is 60.0–90.0 cm^2. The work was carried out in compliance with international ethical standards and rules for working with experimental animals. For the detection of nerve structures of the intestine, a combined intra-extravascular method of impregnation with various silver salts was used (Markov I.I., 2014).

Results: Morphological evidence is obtained for the presence of direct syncytial connections between the neurocytes of the intermuscular and submucous nerve plexuses of the intestines of sexually mature cats and dogs. They are represented by two options: 1) the fusion of the bodies of neurocytes with the formation of binuclear cells and 2) the formation of one common for the two neurocytes of the process. Undoubtedly, synthial neural connections, along with chemical and electrical synapses, make the structural organization of the nervous system more reliable. It can be hoped that the discussion, so emotionally begun by K. Golgi and S. Ramon-i-Cachal and continuing at the present time, will result in the creation of a unified neuron-reticular theory of the organization of the nervous system.

CLINICAL AND IMMUNOLOGICAL EVALUATION OF CHANGES IN THE MEANING OF THE INDICATOR OF IgE IN PATIENTS WITH PAPULES-PUSTULAR SUBTYPE OF ROSACEA, COMPLICATED BY DEMODECOSIS

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A key role in the pathogenesis of rosacea plays a cutaneous inflammatory process. Inflammation is initiated with the participation of TLR2 (toll-like receptors) in keratinocytes. These transmembrane structures can be activated by physical factors (UV, high and low temperatures), a number of antigens (chitinous shell of *D. folliculorum*, glycoproteins *B. oleronius*). In the dermal infiltrates in the lesion are detected in T-lymphocytes, sensitized to antigens *D. folliculorum*, neuropeptides during stress (neuroimmune mechanisms) or the formation of visceral skin reflexes. Further development of inflammation due to the keratinocytes secretion of inflammatory proteases (matrix metalloproteinases, kallikreins) and antimicrobial peptides (alpha-, beta-defensins and cathelicidin LL-37). Currently, the cathelicidin LL-37 plays a significant role in the development of rosacea. This antimicrobial polypeptide consisting of 37 amino acids, belongs to the family of kallikrein-kinin. Its level in the affected skin significantly increased in all subtypes of rosacea. Products of LL-37 is enhanced under the influence of a UV (due to the synthesis of vitamin D), high and low temperatures, infectious agents. Intradermal LL-37 injection induces development dermatitis, clinically similar to rosacea in experimental animals.

This paper discusses the modern view on the problem of the pathogenesis of rosacea: the role of antimicrobial peptides, growth factor of endothelium of vessels, the kallikrein–kinin system, antigen *D. folliculorum*. The role of humoral immunity is evaluated in the form of changes in the indices of IgE in patients with papules – pustular subtype of rosacea, complicated by demodicosis in the acute stage of the disease. The relevance of the study is due to increased incidence, frequent recurrences of the disease, lack of efficiency of existing methods of treatment.

The aim of the study was to evaluate the immune response in patients with papules–pustular form of rosacea, complicated by demodicosis by studies increased IgE. The design of the research included microscopic examination of a scraping with seborrheic zones of the skin, biochemical blood tests, immunological blood tests for IgE by ELISA.

Materials and Methods: Under our observation there were 50 patients with papules-pustular subtype of rosacea, complicated by demodicosis. *D. folliculorum* is confirmed by laboratory tests. All patients underwent immunological examination of blood by ELISA.

Results: Increase in IgE is represented among 50 patients with papules-pustular subtype of rosacea, complicated by demodicosis: in 25 (50%) moderate (120–850 KE/L); 20 (40%) significant increase (1230–5700 KE/L) and only 5 (10%) within the normal range (20–100 KE/L).

Conclusion: The elevated levels of IgE in patients with papules – pustular subtype of rosacea, complicated by demodicosis, is indicative of the role of antigens chitinous shell of *D. folliculorum* in the formation of inflammation, with the participation of TLR2 (Toll-like receptor) in keratinocytes. Study and analysis of the interaction of factors involved in the pathogenesis of rosacea to improve treatment methods and prognosis of disease.

IMMUNOPATHOGENETIC COMORBIDITY IN PATIENTS WITH SEVERE PLAQUE PSORIASIS

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Background: Psoriasis is associated with multiple other comorbidities with a general inflammatory immune component, the most notable being cardiovascular (CVD) and metabolic disorders. But no study has been performed metabolic disorders in Russian population of Psoriasis (PsO) patients. Given the increased prevalence of cardiovascular (CV) and metabolic comorbidities in patients, dermatologists treating psoriasis need to approach the disease as a potentially multisystem disorder. A common mechanism that may explain both psoriasis and atherosclerosis pathogenesis is of great interest and utility. The increase of Th1 and Th17 leading to chronic inflammation is thought to be a patho-denominator for both diseases. In

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addition, adiposity and resultant metabolic syndrome is progressing. ***Objectives:*** To evaluate the prevalence of CV comorbidity, Obs, ThD and DM comorbidity in a hospital-based cohort of patients (pts) with severe PsO.

Methods: 330 pts (234 male (M.)/96 female (F.)), mean age – 39.9±0.9/38.05±1.3 years accordingly, mean PASI 49.4±0.56, PsO duration 11.6±0.6 years were included. PsO pts with endocrine, nutritional and metabolic diseases (E00-E90) (ENMD), including obesity (Obs) and other hyperalimentation (E65-E68), diabetes mellitus (DM) (E10-E14) and PsO pts with cardiovascular disease (CVD), including coronary heart disease (CHD), arterial hypertension (AH), atherosclerosis and cerebrovascular accident (CVA) were identified in the hospital. Database reporting and coding by International Statistical Classification of Disease and Related Health Problems (ICD-10) between 2010–2011 years. M±m, t-test, (%) were calculated. All $p<0.05$ were considered to indicate statistical significance.

Results: 130 out of 330 pts (39.4%) had CVD. CVD coding as I 00–I 99 were found significant often in M. pts compared to F. pts – in 101 out of 234 pts (43.2%) and in 29 out of 96 pts (30.2%) accordingly ($p<0.05$). AH coding as I 10-I 15 were found in significantly more cases in M. pts compare to F. pts - in 90 out of 101 pts (90.1%) and in 24 out of 29 pts (82.7%) accordingly ($p<0.05$). 54 (34 – M./20 – F.) out of 330 pts (16.4%) had ENMD. M. and F. pts were at the same age. Obs coding as E65-E68 were found in significantly more cases in F. pts compare to M. pts – in 13 out of 20 pts (65.0%) and in 19 out of 34 pts (55.9%) accordingly ($p<0.05$). DM coding as E10-E14 were found in significantly more cases in M. pts compare to F. pts – in 16 out of 34 pts (47%) and in 6 out of 20 pts (30%) accordingly ($p<0.05$). No significantly differences were found in the prevalence of CHD and atherosclerosis coding as I 70 between M. and F. pts – in 7 out of 101 pts (6.9%) and in 1 out of 29 pts (4.1%) accordingly ($p>0.05$).

Conclusions: ENMD and CV comorbidities are common for hospital-treated cohort pts with severe plaque PsO. Young M. pts with severe plaque PsO significantly often suffer from DM compared to F. pts. Young F. pts with severe plaque PsO tend to suffer from obesity and other hyperalimentation compared to M. pts. Young M. pts with severe plaque PsO tend to suffer from CVD and AH compared to F. pts. Immune-mediated inflammation is the central actor in atherogenesis beyond all risk factors.

DIABETIC FOOT. CLINICAL AND MICROBIOLOGICAL ASPECTS, TREATMENT STRATEGY

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Diabetes is a significant medical issue associated with the high risk of neuropathies as well as microvascular and macrovascular complications, in particular, diabetic foot. Microangiopathy is reported in 100% of patients (30% of them are the patients with purulent and necrotic complications). This paper highlights major clinical signs of wound infection in diabetics, i.e., muscle weakness, burning feet, pain, reduced touch sensitivity, diminutio and n or absence of peripheral pulses, and lower extremity edema. Microbiological study of diabetic foot lesions is an important step to determine treatment approach. 126 microbial strains were isolated from 97 patients. Major pathogens were gram-positive bacteria (n=103; 82%). Among them, the most common infectious agents were *Staphylococci* (n=68; 65%), i.e., *S. aureus* (n=42; 41%), *Enterococci* (n=17; 17%), *Streptococci* (n=16; 16%), i.e., *S. pyogenes* (n=3; 3%), and *Corynebacterium* (n=2; 2%). In 17% of the patients (n=21), gram-negative bacteria were isolated. Among them, the most common infectious agents were *Enterobacteriaceae* species, i.e., *E. coli* (n=6; 29%), *Klebsiella* (n=4; 19%), *Proteus* (n=3; 14%), *Citrobacter* (n=2; 10%), *Enterobacter* (n=2; 10%), and non-fermenting gram-negative bacteria, i.e., *Pseudomonas aeruginosa* (n=3; 14%) and *Acinetobacter* (n=1; 4%). In 1,5% of the patients (n=2), *Candida* species were isolated. Associations were revealed in 36% of cases. Both gram-positive and gram-negative bacteria were highly susceptible to amoxiclav and cefoperazone-sulbactam. Susceptibility of these bacteria to fluoroquinolones was variable (75–100%); the most effective ones were levofloxacin and moxifloxacin. Intra-arterial levofloxacin (500 mg per day) was administered continuously followed by Polyoxidonium (12 mg per day) through the inserted port-a-cath. 14 patients with purulent necrotic ulcers of foot, toe(s), lower leg, or stump associated with severe pain who were admitted to the surgical department were observed. After 8 to 10 days, the ulcers have cleared up, night time pain and lower leg edema were reduced, body temperature and lab tests have returned to normal ranges. The key measures to prevent diabetic foot are to keep blood sugar levels in control and to start etiologic and antioxidant treatment early.

AGE-RELATED QUANTITATIVE INDICATORS OF GLANDS IN VARIOUS PAGES OF THE VAGINAL VESTIBULE

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The aim of the study was to study the quantitative parameters of small glands in different areas of the vestibule in the different age periods. Macromicroscopic method studied the glands of the vestibule vagina in 69 women of different age. The vaginal area was excised from the corpse by dissection. For the production of the total preparation the resulting material was placed in a 0.5% acetic acid solution with 0.05% methylene blue solution in water (RD Synelnika method). On total preparations, the glands of the vestibule are defined as dark blue formations located on a lighter background of the surrounding wall. The glands are present both in the anterior (closer to the external opening of the urethra), and in the middle and posterior (near to the anus) thirds of the vestibule wall. In the glands, the initial sections and excretory ducts are revealed.

ISSN 1562-3629

On total preparations, the number of glands in different sections of the vestibule vagina was counted. An analysis of the age-related dynamics of this indicator revealed that its value in the anterior third of the vestibular vagina walls in the early childhood is 1.5 times larger ($p<0.05$), in adolescent girls it is 2.0 times larger ($p<0, 05$) and in the first period of adulthood – 2.6 times more ($p<0.05$) than in newborns. The number of glands in the anterior third of the vestibule vagina in elderly women is 1.5 times less ($p<0.05$), in the senile one – 2.0 times less ($p<0.05$) than in the first period of the mature age. In the middle third of the vestibulae vagina in early childhood, the number of glands is 1.6 times greater ($p<0.05$), in adolescent girls – 2.0 times more ($p<0.05$) and in the 1st period of mature age – 2.9 times more ($p<0.05$) than in newborns. The number of glands in the middle third of the vaginal opening in the elderly age is 1.4 times less ($p<0.05$), in the senile one – 1.6 times less ($p<0.05$) than in the first period of adulthood. In the posterior third of the vaginal vestibule walls, the number of glands is 1.5 times higher in early childhood ($p<0.05$), in adolescence it is 1.9 times higher ($p<0.05$) and in the first period of mature age – 2.9 times more ($p<0.05$) than in newborns. The number of glands in the posterior third of the vaginal vestibule walls in the elderly is 1.4 times less ($p<0.05$), than senile age.

POSSIBILITIES OF METHODS OF PHYSIOTHERAPY FOR BRAIN INJURY IN ACUTE PERIOD

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In Russia, the rate of brain injury is 4.5% per 1000 population per year and is one of the top among all causes of primary disability of the adult population. Only 10–20% return to work, about 8% of them retain their professional suitability, 25% need outside help. A patient with a neurological deficit places special obligations on family members of the patient and is a huge financial burden on the health care system. In this regard, it is important to develop methods of treatment that allow victims to be treated as quickly as possible and with the least neurological deficit. The goal is to optimize the treatment of patients with brain injury in the early period due to the additional application of methods of physiotherapy. Materials and methods: 300 patients with brain injury were examined. For 50 people EHF irradiation with a wavelength of 4.9 nm of the VG.14 point was performed for 10 minutes. As a result, improvement of rheological properties of blood was observed, while parameters of acoustic stem evoked potentials were normalized. For 50 people applied electromagnetic waves terahertz range to the traumatic focus of brain damage within 22.5 minutes. At the same time there was an acceleration of the average blood flow velocity along the middle cerebral artery (ultrasound diagnosis) on average by 10%. Patients experienced a faster regression of focal brain changes according to radiation diagnostic methods (CT, MRI). From 3–5 days, depending on the severity of the patient's condition, impulse low-frequency (1 Hz) transcranial magnetotherapy was prescribed, the total exposure time up to 12 minutes. The normalization of the bioelectrical activity of the brain and the improvement of conduction along the corticospinal tract were noted. ***Conclusions:*** Early application of physiotherapy methods allows to reduce the neurological deficit without complications and go to the next stage of rehabilitation.

ADAPTIVE RESTRUCTURING OF THE IMMUNE RESPONSE IN MILITARY PERSONNEL UNDER PROFESSIONAL STRESS

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The study of adaptive shifts in the state of homeostatic systems is of great scientific interest. Post-stress changes in the immune system are a predictor of the formation of immune-mediated syndromes and chronic pathology. Examined 32 military officers (average age 32±5.5 years), performing professional tasks in extreme conditions of hot climate, immediately after the trip, as well as after 6 and 12 months. The state of immune status was assessed by expression CD3+, CD4+, CD16+, CD19+, intracellular content of Foxp3 in CD4+CD25+, the production of granzim in T-lymphocytes and NK cells. After returning from a trip, the examined servicemen showed a persistent tendency to decrease the population of T lymphocytes ($1.7±0.27×10^9$/l before the trip and $1.2±0.13×10^9$/l in the first week after). We observed a statistically significant increase in relative and absolute numbers of B lymphocytes (8±1.16% ($0.15±0.04×10^9$/l) prior to a trip and 19±3.1% ($0.4±0.07×10^9$/l) after returning, $p<0.05$). The activity of antibody production did not change. By the 6th month of observation, the above indicators were returned to the reference limits. Revealed significant decrease of $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory cells ($1.9±0.2×10^9$/l to $1.0±0.18×10^9$/l after a trip, $p<0.05$) and a significant persistent decrease in the number $CD8^+$ lymphocytes expressing the markers of late activation ($CD3^+CD8^+HLADR^+$ $4,2±0,9×10^9$/l to $1.5±0,17×10^9$/l after a trip, $p<0.05$). By 12 months of observation, there was an increase in the number of cells at a late stage of activation ($CD3^+$HLA-DR^+ – 6.55±0.51% in 12 months and 2.35±0.4% before the trip), and increased readiness for apoptosis ($CD3^+CD95^+$ 3.25±0.1% in 12 months and 3.25±0.1% before the trip), the predominance of the processes of late activation of T-helpers ($CD4^+$HLA-DR^+ – 3.35±0.27% in 12 months and cytotoxic lymphocytes ($CD8^+$HLA-DR^+ – 3.2±0.32% in 12 months and 1.25±0.19% before business trip). Thus, the detection of early markers of disintegration of the activity of homeostatic systems of the organism is the basis of preventive and curative measures among persons of dangerous professions.

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ASSESSMENT OF PERSONAL RISKS IN CHILDREN

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Relevance: The personalized approach in creating an individual health profile at the present time is the only acceptable way to avoid the implementation of multifactorial, genetically mediated diseases, such as immunodeficiency, cancer, autoimmune and allergic. The purpose of the formation of personalized risks in children in the first year of life, taking into account changes in the placenta.

Materials and Methods: The distribution of children in the first year of life by groups and placental materials was presented as follows.

1 st gr. – 58 children of the first year of life and placental samples from physiological pregnancy; 2-nd gr. – 50 children and samples of the placenta with the threat of abortion; 3-rd gr. – 52 children and placental samples with gestosis.

Results: Clinical-morphological and immunological comparisons conducted in the study groups revealed the following features of the placenta and risks for the postnatal development of the child.

In group I., pathological changes in the placenta, except involutional, were not recorded. NDP, the parameters of the physical profile, the general resistance in children in the first year were within the age-old norm. Health Group 2A. Allergic diseases were realized in children, physical development parameters were assessed as disharmonious. In the placenta expressed changes in the autoimmune type with a decrease in the mass of the placenta and hemorrhages. Group of health III.

In the third grade, children were treated with frequent and long-term pain. In the placenta, up to 93%, infectious lesions, placental infections, were described. Group of health 2 B.

Conclusion: Thus, changes in the placenta directly reflect the course of the gestational process and can be used as prognostic criteria for postnatal development of the child.

SACCHAROMYCES BOULARDII IN THE TREATMENT OF INTESTINAL INFECTIONS IN CHILDREN

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The most common reason for the development of intestinal dysbiosis in children can be the use of antibiotics, which could lead to antibiotic-associated diarrhea that can be linked to the negative impact of waste products *Clostridium difficile*. The study included 120 children with enteric infections, from them children till 6 months, there were 20 cases (16.7%); from 6 months to 1 year – 60 (50,0%), over the year – 40 (33.3%). The diagnosis of intestinal infection from all the sick children were confirmed bacteriologically: *Ps. aerogenosa* – in 1 (0,8%), *Salmonella enteritidis* – 2 (1,7%), *Proteus vulgaris* – 3 (2,5 %), *Enterobacter cloacae* – 7 (5,8%), *Citrobacter diversus* – 7 (5,8%), *Klebsiella pneumoniae* – 8 (6,7%), *Morganella morganii* – 9 (7,5%), *Proteus mirabilis* – 10 (8,3%), *Citrobacter former* – 12 (10%), acute enteric infection unspecified etiology – in 61 (50.8 percent). The rotavirus antigen in the feces – 12 (10%). Treatment scheme with Enterol (*Saccharomyces boulardii*): for children up to one year 1 sachet 1 time, over one year 1 sachet 1–2 times a day. At admission to the hospital all the examined sick children had a general infectious syndrome in the form of fever, intoxication, decreased appetite, drowsiness, lethargy, hypodynamia. Dyspeptic syndrome was expressed by vomiting, flatulence, rumbling of the abdomen, liquid chair. However, the frequency of clinical symptoms has been mixed. Dyspeptic syndrome in the form of vomiting before treatment was observed in 52 (43.3%) sick children, and after treatment was completely stopped. The flatulence was before treatment in 112 (93.3%) sick children, and after treatment the stomach was soft, painless in all observed children. Thin stool with pathological impurities was before treatment in 109 (90.8%) sick children, and after treatment in 2 (1.7%), ($p \leq 0.05$). The use of Enterol had a positive impact on the duration of basic clinical symptoms in diarrhea. The use of Enterol in the age dosages within 5 days prevents the development antibioticassociated diarrhea in children.

AVAILABILITY OF TORCH-INFECTION IN NEWBORN WITH THE HEMORRHAGIC STROKE

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One of the priority health issues, according to the annual publication of the World Health Organization, "World Health Statistics", which presents statistics on key health indicators, remains the problem of the annual growth of infection by the newborn group of TORCH infections, as well as the growth of childhood strokes up to 1 year of life . The purpose of this study was to investigate the pattern of development of hemorrhagic stroke in children of the first year of life with infection by a group of TORCH infections in the perinatal and postnatal periods of life. The study was subjected to all cases of non-traumatic hemorrhagic stroke in children between 2014 and 2017. From a gynecological anamnesis of 303 women, it was found that pregnancy was accompanied by anemia of pregnant women in 65% (197 women), diseases of the genitourinary system – in 35% (106), circulatory system diseases – in 40.6% (123), venous complications pregnancy – in 22.7% (69), endocrine diseases – in 19.8% (60). Among the infectious factors was infection: cytomegalovirus in 38.3% (116 women), herpes simplex virus – in 44.2% (134), toxoplasmosis – in15.5% (47), chlamydia infection in – 33.3% (101). In the 303

ISSN 1562-3629

children examined, with non-traumatic hemorrhagic stroke, leukocytosis was diagnosed in blood tests with a shift of the leukocyte formula to the left in 57.8% (225 children), thrombocytopenia – in 66.2% (149), anemia – in 41.8% (94). A sharp increase in serum bilirubin was observed in 79% (178), aspartate aminotransferase – in 29.8% (67), alanine aminotransferase – in 80% (180). As a result of the PCR blood test, the following data on the infection of children with previously presented infections in the mother's history were obtained: in 47.2% (143 children) were found to have CMV – 48.2% (146) to HSV, 11.9% (36) – toxoplasmosis and in 29% (88) – chlamydia infection. Also, the results of the study showed that 80.2% (243) had a mix infection: two infections were observed in 39.6% (120), three infections – in 32.3% (98) and four infections – in 7.6% (23). Thus, a correlation was found between the infection of the TORCH infection group and the development of hemorrhagic stroke in children of the first year of life, which requires the personified management of newborns who are at risk.

ANALYSIS OF THE LEVEL OF DISABILITY OF CHILDREN AND ADOLESCENTS DUE TO PATHOLOGY OF THE MUSCULOSKELETAL SYSTEM AND CONNECTIVE TISSUE IN THE VORONEZH REGION, WAYS AND SOLUTIONS

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Children with disabilities have the right to provide orthopedic footwear at the expense of the federal budget in the presence of evidence (998n order of the Ministry of Labor). The support is provided by the disabled child's IPRA. Developed in the MSE bureau. At the same time, the child is examined by MSE medical specialists, the recommendations of orthopedists of medical institutions are taken into account. For 2017 in the Voronezh region 2.250 children and adolescents are recognized as disabled. 537 children out of 1.713 were re-examined and recognized for the first time as a disabled child. At the first time, the category of disabled children up to the age of 18 was established for 36 children and adolescents, which amounted to 6.7% of the total number of newly recognized. In re-examined, the category of disabled children under the age of 18 was established for 427 children and adolescents, which amounted to 24.9% of the total number of re-recognized. Of the total number of children and adolescents with pathology of the musculoskeletal system, 328 people (14.5%) and 309 children and adolescents with paresis of the lower extremities due to diseases of the nervous system (13.7%). In 2017, TCR (orthopedic footwear) was recommended to 568 couples for children and adolescents with musculoskeletal system damage, diseases of the nervous system (cerebral palsy, etc.), and congenital developmental anomalies. By distribution, first recognized as invalids with the pathology of the musculoskeletal system and connective tissue, the absolute number, according to the International Classification of Diseases-10 (ICD-10) (M00-M99), for 2016 in the Voronezh region, were 29 children and adolescents. The proportion of children and adolescents with disabilities with head injuries in the Voronezh Region was 3.9%, while the level of children and adolescents with disabilities with a pathology of the musculoskeletal system and connective tissue in the Voronezh Region per 10 thousand population was 0.76. The absolute number of children and adolescents first recognized as invalids with the pathology of the musculoskeletal system and connective tissue in 2017 is 47 people. The level of such disabled for 10 thousand people in 2016, is 1.20 with a specific gravity of 5.2%. With participation in the MSE of specialists in the profile of disabling pathology, correctly selected TCR for violation of the function of the musculoskeletal system due to diseases and other bone injuries-muscular and nervous systems.

INNOVATIVE TECHNOLOGIES OF REHABILITATION WITH APPLICATION OF MEANS OF INFORMATIONAL MEDICINE

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Rehabilitation activities in chronic intoxications can be resolved with the help of innovative technology using informational medicine (Sidorekno G.I., 1998; 2006, Namazbayeva Z.I., et al. 201. The aim of the research is to study the impact of noncontact electrodynamics vibrowave effect on the human body in chronic intoxications. For relief of chronic intoxication was used electrodynamic vibrowave installation (Namazbaev T.S., Namazbayeva Z.I. “Diagnostic-therapeutic complex”. Patent for invention No. 28386, publ. in bull. No. 6 of 15.06.2016). The groups were selected in women living in the ecological risk conditions on the basis of age stratification and the region equal sampling. Total of 75 people were examined. Voluntary agreement for medical examinations and rehabilitation activities was received. Ultralow concentrations of persistent organic pollutants trigger the mechanisms of endogenous generation of reactive Halogens (AFH) – reactive halogenated compounds (Hal – halogens). Primary products of AFH are catalyzed by the myeloperoxidase (MPO) enzyme with the production of highly reactive hipogalactiei (OHaI-) (Panasenko O.M., et al., 2008; Galijasevic S. 2008). Myeloperoxidase activity in neutrophils of peripheral blood was used as a screening test. Low activity of the enzyme (up to 1.27±0.10 s.u.) was found in 50% of women, which is 54% lower than the limit of physiological fluctuations (2.0–2.8 s.u.). Noncontact electrodynamic vibrowave impact was applied on the liver of patients for 10 minutes, daily for 10 days, with receiving water, obtained by the vibrowave treatment method. At the end of the treatment time, the activity of the enzyme in average was 2.6±1.1 s.u. The activity of the enzyme of two patients increased by 8% compared with physiological indices. The patients themselves noted improvements in overall health, mood, disappeared symptoms of anxiety.

International Journal on Immunorehabilitation
2018 Volume 20 No 2

THE CHANGE IN THE EXPRESSION OF THE NEUROTROPHIC BRAIN FACTOR (BDNF) IN PATIENTS WITH EPILEPSY AND DEPRESSION WITH IMMUNOMODULATION

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At present time, the active attention of researchers is focused on the neurotrophin hypothesis of the depression, the inflammatory theory of epileptogenesis and dysregulation of a brain-derived neurotrophic factor (BDNF). That is induce the decrease in the hippocampal neurogenesis and neurodegeneration associated with epileptogenesis and the development of depression. ***The aim*** is to investigate the concentration of BDNF in blood plasma in patients with epilepsy (PE) and PE with associated depression (PED).

Materials and Methods: The concentration of BDNF was analyzed in blood samples of 60 PE and 38 PED before and after treatment with rIL-2h. The control group is 32 PE only treated AED, and 31 healthy donors (HD).

Results: The initial concentration of BDNF was 4448 pg/l, its concentration is increased to 7023 pg/l ($p<0.01$) in plasma PE, after treatment with rIL-2h. The concentration of BDNF tended to decrease in the control group PE. The average concentration of BDNF has not changed after treatment of rIL-2 in PED, however, within this group was observed two opposite trends of changes in BDNF: in the first subgroup – BDNF increase 2.6 times, in the second – reduction – 3.5 times, after treatment with rIL-2h.

Conclusions. Changes in the expression of BDNF in PE and PED after the treatment of rIL-2h should be considered from the standpoint of neuroplasticity as a predictor of therapeutic response in patients with associated depression.

CORRELATION BETWEEN HUMAN COGNITIVE FUNCTION AND CIRCULATION PROCESSES

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It should be borne in mind the informative assessment of probabilistic prognosis definition as an innovative estimation of human intelligence method which is of interested for a wide range of scientific researches. There are neurophysiological, anthropological, the circulation processes as well as brain nutrition mechanisms studies. The success of solving tasks in situations with varying probability of events and the structure of their relationship determines thuman life quality and safety ultimately. Study of the psychophysiological mechanisms of probabilistic structure formation reflecs the relationship between significant events, in addition to the theoretical interest is obvious of practical value.

APPLICATION OF IMMUNOTROPIC THERAPY FOR PERIODONTITIS

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Background: Modern periodontal therapy is based on the concept of inflammatory bacterial infection, inducing cascade-damaging periodontal tissues proinflammatory cytokines. The results show that the quantitative evaluation of cytokines in the tooth-gum fluid provides useful information for the diagnosis of periodontal conditions. However, the same effect have *Herpes viridae*. Simultaneous local effect on bacterial-viral infection and cytokine cascade can help in the treatment of periodontitis.

Methods: 82 patients with periodontitis and 21 healthy patients were examined *on Herpes viridae, A. actinomycetem comitans, P. gingivalis, P. endodontalis, T. denticola, T. forsythia, P. intermedia, F. nucleatum* in Real-Time PCR method and TNFα, IFNγ, IL4, IL6, IL10, IL18, VEGF cytokines by ELISA method. The standard therapy for periodontitis were included the preparation of care gums "Multifactorial cosmetics. Gel for gums"®, containing VEGF, FGF, EGF, GM-CSG, PGlyRP1 to stimulate dendritic cells and macrophages.

Results: Were EBV, *A. actinomycetemcomitans, F. nucleatum, T. forsythia* were identified in the groups with periodontal disease. There was found correlation between EBV, *P. gingivalis* and IFNγ. After treatment, the level of IFNγ increased from 14.48 to 27.45 pg/ml ($p=0.022$). The study of the level of VEGF before and after therapy with a complex of stem factors also showed a wide spread from 26–279.3 pg/ml before treatment and 16–198.2 pg/ml after treatment. Studies by other authors show that the average concentration of VEGF in the gingival fluid in chronic periodontitis, on the contrary, decreases after treatment. Thus, our data coinside with the data of the authors who showed a decrease in the concentration of VEGF in the gingival fluid during the treatment. In earlier studies, it was shown that the use of local immunotropic therapy reduces the number of representatives of parodontopathogenous microflora. In our study we propose to use IFNγ as an index of the local immune response. Thus, it is possible to use the level of IFNγ in periodontal fluid as an additional laboratory indicator of local inflammation. With the introduction in therapy of the drug with a complex of growth factors and PGlyRP1 the process of healing and elimination parodontogenic microflora was accelerated. Based on the measurement of the level of IFNγ, VEGF and a number of other indicators the screening algorithm for the diagnosis parodontogenic microflora and the monitoring algorithm evaluation of the effectiveness of therapy were developed.

Conclusion: Combination of immunotropic and etiological approach increases the effectiveness of treatment of periodontitis. Developed diagnostic approaches to screening and monitoring help to effectively provide treatment.

ISSN 1562-3629