Societatea de Imunologie din România

INCD "Victor Babeș", Spl. Independenței 99-101, Sector 5, 050096 București

Day of Immunology 2022

Workshop 29 April, 2022

Pediatric Immunology

PROGRAMME

9:00-10:30	
	Opening
9:00 – 9:10	**Day of Immunology** (10 min) **Monica Neagu,** President SIR
9:10 – 9:30	**Adriana Munteanu, PhD student "Recurrent respiratory infections in children – an immunological issue"**
9:30 – 9:50	**Alina Erbescu, PhD student "Immune-related risk gene in autism spectrum disorders children"**
9:50– 10:10	**Mihaela Surcel, PhD postdoctoral fellow "Peripheral immune cellular populations in healthy children – age related differences"**
10:10 – 10:30	**Carolina Constantin, PhD "Immune system ontogeny in humans"**
10:30-10:50	**Lucica Sima "Lysozyme, immune-related molecule in allergic children"**

Extended lymphocyte immunophenotyping for immunodiagnosis of recurrent respiratory infections in the absence of primary immunodeficiency

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Objective: Standard lymphocyte immunophenotyping (SLI) (T-CD3+, T-CD4+, T-CD8+, B, NK cells) contributes to the diagnosis or exclusion of primary immunodeficiencies (PID). In PID-unrelated recurrent infections (RI), SLI may be inconclusive, therefore we investigated some supplementary lymphocyte subgroups with impact in RI pathogenesis: immature B cells (CD19+CD10+), naive B cells (CD19+sIg+), memory B cells (CD19+CD27+), plasma cells (CD19+CD38+), T-double negative (T-DN) cells (CD3+CD4-CD8-), NKT cells (CD3+CD16/56+CD4±CD8±CD1d+). The objective was to guide diagnosis by extended lymphocyte immunophenotyping (ELI), revealing usually untested subgroups that showed significant changes.
Design and method: SLI and ELI was applied in 25 children aged 1-9 years, presenting PID-unrelated RI; control group consisted of 18 healthy subjects. The determinations were made from EDTA-collected fresh whole blood, using 8-color methodology. Data acquisition and analysis of results was performed with Becton-Dickinson equipment: *FACSCanto II* flow cytometer, *FACSDiva 6.1* software.
Results: CD19+ lymphocytes (B cell population) were low in 67% of cases, especially by lowering naive B cells (50% cases). Immature B cells and memory B cells decreased in either 11% cases. CD3+ lymphocytes (T cell population) were low in 11% cases, mainly by decreasing T-CD4+ cells in 28% of cases. T-DN cells were high in 22% of cases, 75% of these being associated with T-CD4+ cell decreases. NK cells were high in 39% cases; NKT cells showed no modification. The overall improvement of ELI was obtained in 22% cases with T-cell modifications and 72% cases with B-cell deficiencies. ELI alone was useful only in 28% patients with B-cell modifications.
Conclusions: ELI determines more accurately the origin of the lowering of different types of lymphocytes in RI, proving usefulness in lowering B and T-CD4+ cells. Diagnosis features can consequently be varied and adapted to each case.

Immune-related risk gene in autism spectrum disorders children

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Autism spectrum disorder (ASD) is a multifaceted neurodevelopmental disorder that has multiple genes deregulations. Lately, immune-related genes have gained attention due to the direct and indirect effect exerted on the neurodevelopmental pathways. The presented paper reviews within autism spectrum disorder, the latest up-dates regarding deregulated genes that encode strictly immune elements, but also genes that encode important players in the immune response. Cytokine genes are the main immune molecules that were found deregulated and strongly associated with neuroinflammation in this disease. Several other immune molecules genes that are involved in antigen presentation and in developing an inflammatory cellular phenotype are described. Oxidative-stress and mitochondrion system genes sustain the pro-inflammatory pattern of these patients. The immune-based gastro-intestinal inflammation pathways are describing many genes that are linked to the immune system sustaining its inflammatory profile. Last, but not least, immune-based epigenetic traits are an expanding domain that awaits its future to shed light on the complex disease that is autism spectrum disorder. Understanding the immune-mediated pathways that influence metabolism, the endocrine, the gastrointestinal system and probably many other systems may lead to novel treatments in ASD.

Peripheral immune cellular populations in healthy children – age related differences

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The child's immune system has a degree of immaturity that is maintained from the new-born to school age. Throughout this period, the normal child may contract a number of infections - mostly subclinical - that help strengthen the immune system, expand the immune memory and increase the efficiency of the immune response, conditions that will help the immune system to mature in the prepubertal period.

The researches into recurrent infections in children is trying to determine the "normal" number of infections a child can get during the first 14 years of life. It was concluded that they are more common in the new-born and infant phases, decreasing in number and severity as they age.

If repeated infections with significant clinical expression and prolonged or complicated evolution occur during childhood, the existence of an immunodeficiency (ID) with a humoral or cellular mechanism should be considered. Much more common than primary ID (IDP) are recurrent, less severe infections that occur in the absence of IDPs, in conditions of an immune system without major apparent deficiencies.

The immunological investigation of a recurrent infection syndrome is now relatively clear: the diagnosis or exclusion of an IDP. If IDP has been ruled out, given that immunological tests do not reveal a molecular deficiency or major deficiency, a number of diagnostic difficulties arise, which requires further investigation. This refers to a number of cell categories that may have an impact on the pathogenesis of recurrent infections.

Thus, in the present study, sets of normal values were established for the following cellular sets and subsets:

- B lymphocytes (total B lymphocytes, immature, naive, memory B cells) and plasma cells, based on surface markers CD45, CD19, CD20, CD27, CD10, CD38, sIgM, sIgD;
- T lymphocytes (total T lymphocytes, T helper, T suppressor / cytotoxic, double negative T cells, regulatory T cells) and NK cells (total NK and NKT) based on surface markers CD45, CD3, CD4, CD8, CD1d, CD16, CD56.

The determinations were performed by flow cytometry, and the sets of normal values obtained were reported at significant age ranges, constituting an important reference system for immunological investigation in children.

Immune system ontogeny in humans

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The immune system of a child has a degree of immaturity that is maintained from the time the child is a new-born until 6-7 years of age. This immaturity may be due to age-related functional disorders in the immune response. Therefore, the child's immune response is considered 'hypo-inflammatory'. Throughout this period, a healthy child can contract a series of infections, which contribute to the strengthening of the immune system, expanding immunological memory and increasing the immune response. These conditions contribute to the maturation of the immune system during the pre-pubertal period. The immune system suffers major changes throughout life. There are over 1600 genes that are activated/suppressed during activation/control of innate and adaptive immune responses. The genes that encode immune-related elements and the transcriptomic apparatus that regulates these genes have to continuously adapt to changes in the external and internal environment. For humans, the immune system is immature at birth and hence will evolve during childhood, as being exposed to various new antigens. At birth and several months after, the immune system is represented in majority by the maternal antibodies. Afterwards, as the immune system is becoming more mature, the innate and the adaptive arms of immunity evolve and maintain an increased strengts up to around 15 years where it enters in a plateaux that will decline afterwards in older ages.

Lysozyme, immune-related molecule in allergic children

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Lysozyme (muramidase), is a functional molecule that has a clear antimicrobial function being an enzyme produced by the cells of the innate immune system arm. Lysozyme attacks peptidoglycans that are the major component of gram-positive bacterial cell wall, inducing hence the loss of bacterial cell walls integrity and further bacteria lysis. Lysozyme is abundant in secretions (e.g. tears, saliva, human milk, mucus) and is present in the cytoplasmic granules of macrophages and polymorphonuclear neutrophils. Large amounts of lysozyme are found in egg white. Hen egg white lysozyme has a high thermal stability in comparison to the human milk lysozyme that loses its activity quickly upon temperature raise.

Evaluation in allergic children the mapping of epitope-specific (ses-)IgE and ses-IgG4 was done. The results show that epitopes from ovotransferrin, lysozyme, albumin, vitellogenin-II fragment, and vitellogenin-1 precursor were found. Actually, allergenic peptides comprised a batch of over 180 de peptides. Out of this peptide's batch, in allergic children the main ses-IgG4 and ses-IgE were raise against ovomucoid and ovalbumin.