SUMMARY

Allergic contact dermatitis (ACD) is a chronic, inflammatory skin disease that occurs in 15%-20% of the general population. This disease is an allergen-specific delayed hypersensitivity reaction of type IV according to Gell and Coombs classification. The subpopulations of T-lymphocytes: $CD8^+$ (cytotoxic T cells, Tc) and $CD4^+$ (helper T cells, Th) play a main role in the pathogenesis of allergic contact dermatitis. The importance of regulatory T cells (Treg) has been underlined. The recent years studies have also indicated the participation of innate immunity mechanisms and cells such as neutrophils and monocytes. Most often the eczematous lesions develop in the place of contact with the alergen and the adjacent area. Skin lesions in ACD can also arise in distant areas, creating an image of a disseminated contact dermatitis. Mechanisms responsible for the course of ACD remain incompletely defined. Recently toll-like receptors (TLR) and osteopontin (OPN) have been shown to play a role in the pathogenesis of allergic contact dermatitis.

Toll-like receptors are glycoproteins expressed on the surface of immune cells, including monocytes and neutrofils. These receptors are the elements of innate immunity and are also responsible for initiation of adaptive immune response. The role of toll-like receptors in the development of allergic contact dermatitis is poorly understood. The study in mice have shown the ability of TLR2 and TLR4 to bind fragments of endogenous extracellular matrix (ECM) and stimulate the immune response without the participation of the infectious agent. It can be important in development and course of inflammatory reaction in allergic contact dermatitis.

Regulatory T cells constitute a population of $CD4^+$ lymphocytes. They participate in maintaince of the tolerance to self-antigens. They also suppress the immune response by inhibiting the activation, proliferation and function of $CD4^+$ and $CD8^+$ effector cells. In recent studies the role of regulatory T lymphocytes in allergic contact dermatitis has been found, however, the function and mechanisms by which the Tregs regulate the inflammatory process in the skin are poorly understood. Increased number of Tregs has been shown in the skin inflammation suggesting that regulatory T lymphocytes are involved in the suppression of the inflammatory reaction in ACD.

Osteopontin is a phosphorylated acidic glycoprotein produced by many cells of the immune system such as lymphocytes, macrophages and dendritic cells. Osteopontin occurs in two isoforms: secreted OPN (sOPN) and intracellular OPN (iOPN). In the skin of mice with

eczema lesions the increased sOPN expression, produced by keratinocytes and effector T lymphocytes has been found. In addition, serum sOPN concentration have been shown to increase in patients with acute stage of allergic contact dermatitis.

The aim of the study was to determine the expression of TLR2 and TLR4 on monocytes and neutrophils, to assess of the T lymphocyte subsets: $CD4^+$, $CD4^+CD25^+$, $CD4^+CD25^{high}CD127^{low}$ and $CD4^+$ T lymphocytes producing intracellular osteopontin in the blood of patients with allergic contact dermatitis.

Fifty-nine adult patients with disseminated form of allergic contact dermatitis and 46 healthy controls were enroled into the study. Diagnosis of allergic contact dermatitis was established on the base of medical history and the characteristic morphology of skin lesions. The extent and severity of skin lesions were assessed using the EASI index (eczema area and severity index). In patients with allergic contact dermatitis the blood samples were taken twice: in the acute stage of the disease and in the remission. The expression of TLR2 and TLR4 on monocytes and neutrophils was assessed in group I (33 patients and 26 controls). T lymphocytes producing intracellular osteopontin were examined in group II (26 patients and 21 controls). In both groups examined subpopulations of T lymphocytes: $CD4^+$, $CD4^+CD25^+$ and $CD4^+CD25^{high}CD127^{low}$ were assessed. The expression of TLR2 and TLR4 receptors on monocytes and neutrophils, determination of $CD4^+$, $CD4^+CD25^+$ and $CD4^+CD25^{high}CD127^{low}$ T lymphocyte subsets and T lymphocytes with expression of intracellular osteopontin were analyzed by flow cytometry (Cytomics FC500, Beckman Coulter).

The results of the study showed a significantly increased percentage of the monocytes with expression of TLR2 and TLR4 in peripheral blood in patients with acute ACD compared with patients in remission and healthy persons. An increase of the percentage of activated $CD4^+$ T lymphocytes with the $CD4^+CD25^+$ phenotype and a reduced percentage of regulatory T lymphocytes defined as the $CD4^+CD25^{high}CD127^{low}$ subpopulation were also found in the patients with acute stage of allergic contact dermatitis. The percentage of $CD4^+CD25^+$ T lymphocytes showed a positive correlation with the EASI index. Patients with the acute form of ACD showed significantly higher percentage of $CD4^+$ T lymphocytes with expression of intracellular osteopontin compared with healthy controls. An increase in blood population of the T lymphocytes with expression of iOPN persisted during remission of the disease.

The results suggest that in the acute stage of allergic contact dermatitis blood monocytes are activated via TLR2 and TLR4. During remission of disease there is no factor activating these cells or undetermined factors that inhibit their function are secreted.

The decreased percentage of regulatory T lymphocytes in the blood of patients with acute ACD in comparision with results of healthy group may be related to the transformation of Tregs into $CD4^+CD25^+$ T cells, of which increased percentage was found in acute stage of contact dermatitis. It may also indicate their increased recruitment to the skin. The positive correlation between the percentage of $CD4^+CD25^+$ lymphocytes and the EASI index may be indirect evidence for the importance of activated lymphocytes with the $CD4^+CD25^+$ phenotype (in addition to $CD8^+$ lymphocytes) as effector cells in allergic contact dermatitis.

The increase in the T cell population with intracellular expression of osteopontin can indicate its participation in the acute ACD. Taking into account previous studies showing an increase sOPN level in the serum of patients with acute ACD could indicate the origin of sOPN from blood lymphocytes and the possibility of transformation of iOPN into sOPN. These results can also confirm the pro-inflammatory effect of OPN in the acute ACD. An increased percentage of T lymphocytes with iOPN expression during remission of skin lesions can indicate the prolonged presence of these cells in the blood, however, there is no OPN secretion from T lymphocytes.