ImmunoTools *special* Award 2014

Anna Strzepa, PhD-student

Supervisor: Prof. Dr. Marian Szczepanik

Medical Biology Department, Jagiellonian University Medical College, Kopernika 7, 31-034 Kraków, Poland

Gut microbiota modulates course of contact sensitivity (CS) in mice

Studies conducted over many years have shown that the bacteria living in the alimentary tract have an essential role in the processes of food digestion, maintaining homeostasis, modulating lipid metabolism, promoting angiogenesis, supporting the immunity to infections and development of the immune system. Current reports also show that microbiota plays a crucial role in shaping the immune response. It becomes increasingly clear that less exposure to microbiota may contribute to the observed rise in the "immune-mediated" diseases as well metabolic disorders, and potentially, neoplastic diseases. The advances in medicine in the field of infection therapy promoted an increasing application of antibiotics, which apart from eliminating pathogens also partially eliminate the natural human bacterial flora. In addition, antibiotics are ubiquitously present in the food, which also can affect microbiota composition. It has been observed that alerted bacterial profile, known as dysbiosis precedes development of allergy in children later in life including atopic dermatitis. Another form of skin disease with an underlying hypersensitivity reaction is contact sensitivity (CS) to haptens known in humans as allergic contact dermatitis. It should be noted that the contact dermatitis developed as a result of exposure to chemical substances in the workplace, constitutes about 30% of all occupational diseases and represents a severe social and economic problem essentially affecting a patients' quality of life and hence their capacity to work. At present there is no unequivocal information on the effects of natural gut flora on the course of CS response. Therefore, given the widespread therapeutic application of antibiotics, and progressive "chemicalization" of the environment entailing enhanced exposure e.g. to haptens, studies concerning the partial elimination of the natural gut flora on CS response seem appropriate.

The aim of our study is to determine the influence of antibiotic induced partial gut flora depletion on CS in mice.

Our work showed that oral treatment with broad spectrum antibiotic prior to hapten sensitization inhibits CS response in mice. Suppressed CS reaction *in vivo* correlates with reduced number of both aerobic and anaerobic bacteria in the colon content. Antibiotic induced suppression of CS was reversed after reconstitution with normal gut flora. Adoptive cell transfer experiments showed that gut flora modification with antibiotic induces regulatory cells in axillary and inguinal lymph nodes (ALN), spleen (SPL), mesenteric lymph nodes (MLN) and Payer patches (PP).

In the proposed study mice will be orally treated with water alone or water with antibiotic for two weeks prior to hapten sensitization. Four days post immunization proper lymph organs will be isolated. To identify cell populations responsible for antibiotic induced suppression of CS response, cells from ALN, SPL, MLN and PP will be stained with proper ImmunoTools anti-mouse antibodies for flow cytometry (CD4, CD8, CD8a, CD11b, CD19, CD25, CD40L, CD80, CD86, B7-1, IFN-gamma, IL-2, IL-4, IL-10, IL-12p40, Gr-1, NK-cells, TCRα/β, TCRγ/δ).

It is well known that dendritic cells (DC) are conductors of the immune response. Therefore, we would like to test ability of DC from naïve and antibiotic treated mice to convert naïve T cells to various T cell populations. To perform *in vitro* differentiation studies, 10^6 of $CD4^+$ $CD62L^+$ SPLC will be stimulated with anti-CD3 and anti-CD28 mAbs and proper recombinant mouse cytokines from ImmunoTools for Th1 (IL-12, IFN-γ, anti-IL-4), Th2 (IL-4, anti-IFN-γ), Th17 (IL-6, TGF-β, IL-1β, IL-23 and anti-IL-4, anti-IFN-γ), Treg (TGF-β, IL-2, anti-IL-4, anti-IFN-γ). 72 h later supernatants will be tested for IL-4, IL-10, IL-17A, TGF-β, IFN-γ concentration by ELISA. Cells will be stained for the expression of surface markers with ImmunoTools anti-mouse antibodies (TCRβ, CD4, CD25), transcription factors (T-bet, GATA3, RORα, RORγt, FoxP3) and intracellular cytokines (IL-4, IL-10, IL-17A, IFN-γ) and analyzed by flow cytometry.

We believe that ImmunoTools antibodies and recombinant mouse cytokines will help us to investigate mechanisms involved in antibiotic induced suppression of CS reaction in mice.

ImmunoTools *special* AWARD for **Anna Strzepa** includes 21 reagents

FITC - conjugated anti-mouse CD3, CD25, CD45R(B220), TCRγ/δ, CD44,

PE - conjugated anti-mouse CD8, CD8a, CD62L, CD134, Gr-1, TCRα/β,

APC - conjugated anti-mouse CD4, CD11b, CD19, NK-cells,

recombinant mouse cytokines: rm IL-4, rm IFN-gamma, rm IL-6, rm IL-1beta, rm IL-2, rm IL-10

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