*Review paper*

# Immunostimulatory effects of lipopolysaccharide

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***Abstract***

*The lectin mitogens Con A and PHA administered in vitro stimulate preferentially proliferative response of T lymphocytes. The response is accepted as a reflective for feature of immune competence of responding cells. The accessibility of specific phytomitogens for experimental determination of T lymphocyte properties creates a comfortable investigative circumstances. The authors explain why the nature of the population of B lymphocytes excludes the existence of a similar specific universal experimental tool, which could reveal complex immune properties of the population. The mechanisms of in vitro influence of putative candidates for being the B cell mitogens (PWM, LPS) are shortly presented and discussed. On the grounds of these data authors suggest that no PWM nor LPS can be estimated as a specific and selective mitogens for B lymphocytes.*

***Key words:*** *LPS, why specific B cell mitogen does not exists?*

*(Centr Eur J Immunol 2011; 36 (2): 85-86)*

Lipopolysaccharide (LPS, endotoxin) the membrane constituent of Gram-negative bacteria, is an immunostimulator commonly used in different *in* vitro experiments investigating effects of tested agents on function of activated immune cells. The first step in this kind of experiments is to introduce the cells into active state which can be demonstrated by proliferation and/or production of cytokines, monokines or specific immunoglobulins. Depending on the type of cells which are chosen to be the target of initial stimulation, already known different immunostimulators or mitogens could be used. The question is what a place the LPS occupies on the list of known mitogens or immunostimulators and which type of immune cells preferentially react in response to this agent?

Among several mitogens (agents able to induce polyclonal proliferation of defined type of cells) the best known are the lectins: concanavalin A (Con A) and phytohaemagglutinin (PHA). The both mitogens stimulate preferentially proliferation of multipotent T lymphocyte population discriminating between the fresh thymic emigrants and young, mainly naive T cells (ConA) and matured peripheral T memory cells which respond more vigorously to PHA. Determining *in vitro* ability of tested immunocyte population to respond to optimal doses of PHA and ConA one can estimate if the population contains the proper proportions of the young and matured lymphocytes. For example, for the human mononuclear peripheral blood cells (PBMC) the response to PHA should be twice as high as the response to Con A measured by 3H-tymidine uptake during the last 18 hours of the three-days cultures [1, 2]. Moreover, on the grounds of response to Con A and/or PHA many other important T cells features can be determined, e.g. suppressive activity of T regulatory cells, influence of monokines on T cell response, and finally, qualitative and quantitative estimation of the production of a full range of different cytokines [3-6]. The accessibility of specific phytomitogens in experiments aimed to determine the T lymphocyte properties create a comfortable investigative circumstances.

Is the same or similar situation related to the investigation of the properties of B lymphocytes, monocytes or macrophages? Are we in disposal of specific B cell mitogen? To answer the question one has to remind the differences between T and B cells. The former are mainly initiators and regulators of immune response induced by MHC-restricted manner and their functions are almost

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always preceded by cellular proliferation. In contrast to that, the later one (B cells), similarly to the monocytes and macrophages, belong to the group of antigen presenting cells (APC) with multiple and different effector functions (e.g. phago- and pinocytosis, antigen presentation in MHC-restricted manner, immunoglobulin and cytokine production, cytotoxicity), and their proliferation, if any, in response to external signals is not the most significant feature.

For measurements of proliferative abilities of B cells the pokeweed mitogen (PWM) has been temporarily in use with believe of its specificity until the time when its mitogenic influence appeared to be indirect employing the primary participation of TCD4 lymphocytes.

B lymphocytes can be stimulated by LPS by Toll-like receptors, which are not exclusive feature of these group of lymphocytes but are also present on many other types of immune cells active in innate immunity [7, 8]. Therefore, B cells responding to LPS take only part in the response of many other cells (e.g monocytes, macrophages, dendritic cells) which, in turn, by influence of monokines and cytokines may affect behaviour of TCD4, TCD8, B, and NK cells. LPS, therefore, can not be discerned as a selective and specific B lymphocyte stimulatory agent. The way of B cell stimulation by LPS starts from signaling via TLR4/MD2 complex, which in cooperation with another member of Toll-like receptor, RP-105/MD1 (CD180), may lead to proliferative response, class switching, differentiation into plasma cells and antibody production [9, 10]. Lymphocytes lacking of CD180 are deeply impaired in reactions to LPS. Several other factors are required for B cell response to LPS. The guanine nucleotide exchange factors Vav1 and Vav 2 regulate the way of response in terms of proliferation or immunoglobulin production. While TLR4 is expressed on various types of immune cells, CD180 characterizes mature B lymphocytes. They can be activated by LPS if tyrosine phosphorylation of CD19 occurs following by the formation of the complex chain LPS/CD180/CD19/Vav/Lyn [8, 10-13]. Deficient expression of CD19 inhibits B cell response to LPS.

While proliferative response of T lymphocytes to T-cell specific phytomitogens can be estimated as an expression of their immune competence, no similar experimental tool exists, specific for B lymphocyte, which could reveal complex immune properties of the population. In the front of numerous functions of B lymphocytes as an antigen presenters, cytokine producers, precursors maturing into plasma cells and producers of 5 class immunoglobulins, their insignificant proliferative response induced, for example, by PWM or LPS, if measured in population containing different types of cells (e.g splenocytes, PBMC), is not enough to characterize the immune potential of this unique cellular population.

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