



Review

Interleukin 10 and Its Role in the Regulation of the Cell-Mediated Immune Response in Syphilis

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Abstract. Data concerning interleukin 10 (IL-10), a cytokine of Th2 lymphocytes, and its inhibition of Th1 lymphocytes from secreting IL-2 and interferon (IFN) are presented. It has been indicated that IL-10 also inhibits other cells from producing IL-12 and nitric oxide (NO). It is known that all these factors take part in the cell-mediated immune response and immunity. This inhibition may facilitate the multiplication of *Treponema pallidum* and the development of disease despite the presence of immunologically competent cells. It has also been demonstrated that in late latent syphilis, when Th1 lymphocytes are not able to produce IL-2 and IFN, the cells are able to produce only IL-12 and NO. This fact seems to suggest that these factors take over the immune function when cells are stimulated again by *treponemes* which, after many years of latency, begin to multiply. Thus, a high level of IL-12 and NO seems to be an indicator of the development of the third stage of disease.

Key words: IFN; IL-2; IL-10; IL-12; nitric oxide; cell-mediated immune response; syphilis.

Introduction

Interleukin 10 (IL-10) is a factor which takes part in the regulation of cell-mediated immune response and immunity¹. This cytokine was first identified in 1989 as a product of mouse Th2 clones which inhibits interferon γ (IFN- γ) secretion. IL-10 is an 18 kDa cytokine produced by the Th2 subset of CD4⁺ helper lymphocytes, but it is not a strictly Th2-specific cytokine^{1, 23}. In humans, CD4⁺ T cells and clones of the Th0, Th1 and Th2 subsets produce IL-10 on antigenic stimulation. It is also produced by activated macrophages, some activated B cells, and some non-lymphocytic cell types (e.g. keratinocytes)^{23, 34}. The two major activities of IL-10 are to inhibit cytokine (i.e. TNF, IL-1, IL-12 and the chemokines) production by macrophages, IFN- γ production by Th1 cells, and to inhibit the accessory

functions of macrophages in T cell activation^{9, 10, 32}. This latter effect is due to a reduced expression of class II MHC molecules^{6, 8, 11}. In addition to its inhibitory effects on macrophages and lymphocytes, IL-10 has stimulatory actions on B cells. It may be a switching factor for the production of IgG4 in humans^{1, 23, 31}. Interestingly, the genome of the Epstein-Barr virus contains a gene homologous to IL-10¹.

The Dependence between the Ability of Th2 Lymphocytes to Secrete IL-10 and the Inhibition of Th1 Lymphocytes from Producing IL-2 and IFN in Syphilis

The results of our previous work indicated that the cell-mediated immune response, of importance in the

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protection against *Treponema pallidum*, is distinctly inhibited in some stages of syphilis^{3, 20, 26}. It was found as a diminished ability of lymphocytes to produce migration inhibition factor (MIF), which affects various anti-microbial functions²², and anti-treponemal limphotoxin²⁷, which kills treponemes both *in vitro*^{12, 29} and *in vivo*²⁴. A distinct weakening of the ability to mount a delayed-type hypersensitivity reaction in rabbits infected with *T. pallidum*²⁵ was also found. The suppression of the cell-mediated immune response, which assumes the main role in immunity against *T. pallidum* infection, seems to be not only a cause for the development of the disease, but also a reason for the difficulties in curing the late stages of syphilis despite proper treatment. Considering that patients with early syphilis, as opposed to latent disease, are easy to cure, the idea emerged among scientists to examine if and to what degree the immunological response facilitates treatment. Because IL-2 and IFN- γ , cytokines of Th1 lymphocytes, are very important for the cell-mediated immune response, we have taken into account the ability of syphilitic patients' cells to produce them²⁸. Recent data have indicated that the ability of lymphocytes to produce IL-2 and IFN- γ is also distinctly suppressed at some stages in syphilis²⁸. A strong correlation between high IL-10 levels and low IFN- γ , IL-2 and even TNF- α levels was also observed. The data suggested that IL-10 inhibits the ability of Th1 cells to secrete cytokines in syphilis. Such inhibition has also been observed in some other disease²¹. The role of IL-10 as a regulator of Th1 lymphocytes' ability to produce cytokines seems to be useful in some diseases, because high levels of Th1 cytokines in an organism, particularly in autoimmune diseases or transplant rejection, may be harmful²³, though this is not the case in syphilis²⁸.

Our latest studies on the mechanism of the cell-mediated immune response and immunity in syphilis were performed in all stages of disease²⁸. A correlation was found between the high ability of Th1 lymphocytes to secrete cytokines and the healing of syphilitic lesions. The data indicated that these cytokines (IL-2 and IFN- γ) may take part in immunity. The correlation was observed in primary seropositive and secondary syphilis, stages of the disease when the ability of Th2 lymphocytes to secrete IL-10 was distinctly low (Fig. 1). In the next stages of syphilis, especially in early latent syphilis, the ability of Th2 lymphocytes to secrete IL-10 grows, but the ability of Th1 lymphocytes to produce IL-2 and IFN- γ is distinctly diminished. It is known that IFN- α , - β and - γ are produced in syphilis, but the highest level was of IFN- γ . In the late stages of

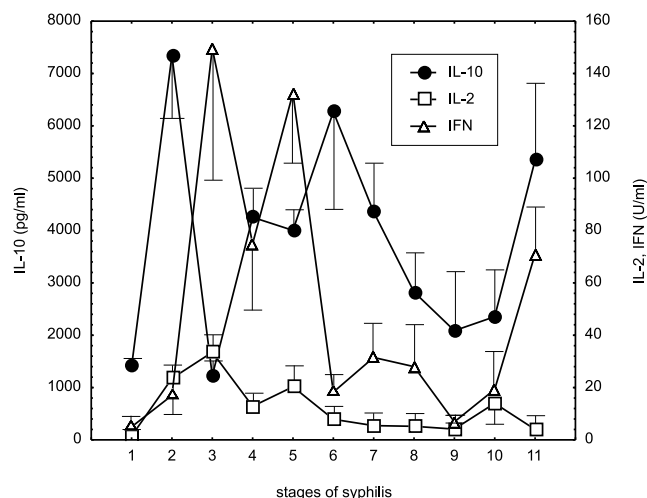


Fig. 1. Dependence between the ability of cells to produce IL-10, IL-2 and IFN after stimulation with *Treponema pallidum*. To determine the level of IL-10 in cell culture supernatants, an ELISA test was performed using Pharmingen (San Diego, USA) reagents and procedure. IL-2 and IFN activities in culture supernatants were measured by biological methods using IL-2-dependent CTLL-2 and A549 cell lines. The proliferation/death of cells was determined by a colorimetric method using MTT²⁸. 1 – control, stages of syphilis: 2 – *Lues I seronegativa*, 3 – *Lues I seropositiva*, 4 – *Lues II recens*, 5 – *Lues II recidivans*, 6 – *Lues latens recens*, 7 – *Lues latens tarda*, 8 – *Lues nervosa asymptomatica*, 9 – *Paralysis progressiva*, 10 – *Lues cerebrospinalis*, 11 – *Tabes dorsalis*

disease, with the exception of cerebrospinalis syphilis, the increased ability of cells to produce IL-10 was accompanied by a very weak ability to secrete IL-2. Similar results were obtained when the ability of IL-10 production was compared with that of IFN. Production of both IL-10 and IFN was found only in tabes dorsalis²⁸. These results shed some light on the mechanism of the immunological response. They also seem to explain why syphilis develops despite the presence of immunologically competent cells.

In light of the above data, the cytokines of Th1 lymphocytes may stimulate the cell-mediated immune response and, in this way, contribute to protection against *T. pallidum* infection. The same conclusion was drawn by ARROLL et al.², who indicated that Th1 lymphocytes are dominant in the cellular infiltration of the syphilitic lesions in rabbits, and that the cytokines of the Th1 phenotype play a key role in the clearance of *T. pallidum* from infected tissue.

The suppression of the Th1 lymphocytes' ability to produce cytokines and weakening of the cell-mediated immune response and immunity in late syphilis may be the reason why patients in these stages of disease are difficult to cure. This suggests that immunological response simplifies therapy.

The Influence of IL-10 on the Ability of Cells to Produce IL-12 and Nitric Oxide, Factors Important for Immunity

IL-12 shows a strong synergy with IL-2^{14, 23} and takes part in the response to bacterial, parasitical and viral products³³. IL-12 plays a central role in macrophage – T cell interactions and in protective cell-mediated immunity against intracellular microbes²³. It has been identified as a cytokine produced by B cell lines and infected phagocytic cells which stimulates the production of IFN- γ by natural killer (NK) cells and cytotoxic T lymphocytes. IL-12 enhances the cytotoxic activity of both CD8⁺ T cells and NK cells^{23, 33}. It is also known that IL-12 is an inducer of Th1 lymphocyte differentiation; thus, IL-12 is a key initiator of cell-mediated immunity²³.

The lack of information about its role in syphilis inspired the interest in examining whether this cytokine is produced in *T. pallidum* infection. The question also arose as to whether its secretion is regulated by IL-10¹⁷.

It was found that in the primary and secondary stages of syphilis, IL-12 is produced with the same dynamics as the Th1 cell cytokines¹⁷. These data suggested that IL-12 takes part, similarly to the cytokines of Th1, in immunity against *T. pallidum* infection. It was also found that the ability of cells to produce IL-12 was suppressed when IL-10 secretion increased. This was especially seen in early latent syphilis. The data indicated that the cells regulation by IL-10 involved not only the secretion of cytokines produced by Th1 lymphocytes, but also by other cells. In late latent syphilis, when lymphocytes produce IL-10 very weakly, the ability of cells to produce IL-12 increases. A high level of this cytokine in late latent syphilis seems to suggest that the multiplication of treponemes begins and the tertiary stage of disease develops. At this stage of syphilis, cells are also producing MIF and, as previously indicated, nitric oxide (NO)¹⁸. The data indicated that the inability of Th1 lymphocytes to produce IL-2 and IFN, important for immunity, in late latent syphilis is replaced by other cells which are able to produce MIF, IL-12 and NO. These might be B lymphocytes, macrophages, dendritic cells, nerve cells and others.

NO is responsible for the intracellular destruction of microorganisms by macrophages and also for the killing of neoplastic cells. The synthesis of NO by activated macrophages correlates with their cytotoxicity. Different tissues are able to synthesize NO, including cells of the immune system¹⁹. Cytokines such as IFN- γ and TNF- α play an important role in the regulation of NO synthesis. Both these cytokines are produced in

syphilis^{28, 30}. It is known that IFN- γ synergizes with TNF- α in the production of NO by macrophages^{16, 19}. Moreover, IFN- α and IFN- β can stimulate its production⁷. Other cytokines, such as IL-4, TGF- β , IL-10 and IL-8, inhibit this process^{4, 5, 13, 15}. The biosynthesis of NO is an enzymatic process. NO is produced from L-arginine as a very unstable product which is oxidated to nitrites and nitrates¹⁹. The determination of NO as NO₂⁻ or NO₃⁻ was used as an indicator of the bactericidal activity of macrophages and to estimate the function of macrophages and other cells in diseases. NO was also examined to determine the ability of the cells of syphilitic patients to produce it¹⁸.

It was found that the cells of syphilitic patients with different stages of disease were able to produce this factor. NO is produced intensively in primary and secondary syphilis, with distinct clinical symptoms. The greatest ability to produce this factor was found in late latent syphilis, where, after many years of latency, syphilis develops again. In very late syphilis the cells' ability to produce NO was already weak. In these stages of disease, this low ability of cells to produce NO may delay the development of the late symptoms in which NO may take part. Of the two cytokines produced by Th2 lymphocytes, namely IL-6 and IL-10, only IL-10 distinctly inhibited NO secretion¹⁸.

Summarizing the above data, it is worth emphasizing that only IL-12 and NO are intensively produced in late latent syphilis (Fig. 2). In view of these data, we

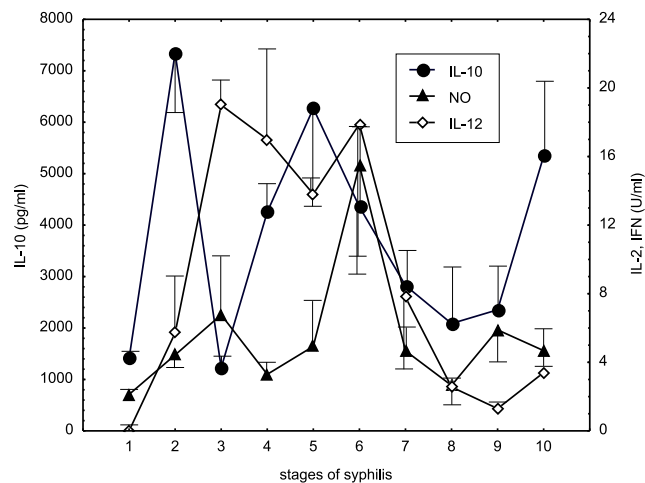


Fig. 2. Dependence between the ability of cells to produce IL-10, IL-12 and NO after stimulation with *Treponema pallidum*. To determine the levels of IL-10 and IL-12 in cell culture supernatants, an ELISA test was performed using Pharmingen (San Diego, USA) reagents and procedure¹⁷. To examine the level of NO as NO₂⁻, Griess reagent was used¹⁸. 1 – control, stages of syphilis: 2 – Lues I seronegativa, 3 – Lues I seropositiva, 4 – Lues II recens, 5 – Lues latens recens, 6 – Lues latens tarda, 7 – Lues nervosa asymptomatica, 8 – Paralysis progressiva, 9 – Lues cerebrospinalis, 10 – Tabes dorsalis

propose to draw attention to these two factors as indicators of the development of the late stages of the disease. In late latent syphilis, after many years of duration, treponemes again begin to multiply and stimulate cells other than Th1 lymphocytes to immunological response. Though some immunological response exists, the patients are, as mentioned above, very difficult to cure. This suggests that only the cytokines of Th1 lymphocytes and antibiotics are able to remove the bacteria from the organism. It is known that in the primary and secondary stages of syphilis, when Th1 lymphocytes are able to produce cytokines (IL-2, IFN) and when other cells are producing IL-12 and NO, the patients are easy to cure. So the results of our previous research^{17, 18, 28} support the suggestions of others that only a properly functioning immunological system in co-operation with antibiotics is able to cure patients from syphilis²⁶.

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