Chapter VIII
Case Report

The Singularity of Il-12 in the Cytokine Network

Paolo Lissoni*, Franco Rovelli, Vezika Cenaj, Arianna Lissoni, Giusy Messina, Giuseppe Di Fede

Insititute of Biological Medicine, Milan, Italy

***Corresponding author:** Paolo Lissoni, Insititute of Biological Medicine, Milan, Italy, E-mail: paolo.lissoni@gmx.com

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Abstract

The discovery of cytokines has completely modified the approach to the Immunology and to the treatment of human systemic diseases due to immune alterations. However, because of the multiple and often contraduictopry effects of the single cytokines, the clinical use of cytokines in the treatment of human diseases is stillat the beginning. Since the immune responses depend on the interactions between the innate and the adaptive immunity, it is possible to identify within the great number of cytokines the singularity of IL-12, which may be considered as the link between innate and adaptive immunity, as shown by the ability of IL-12 to promote IL-2 secretion from TH1 lymphocytes, to activate dendritic cell functions, to stimulate the cytotoxicity mediated by both NK cells and T lymphocytes, and to inhibit T reg cell functions. Moreover, IL-12 would represent one of the main antitumor cytokines in humans. The antitumor activity of IL-12 is due to several mechanisms, including stimulation of IL-2 secretion, inhibition of T reg system, anti-angiogenic action, stimulation of cytotoxic T lymphocytes, and modulation in an antitumor way of the macrophage activities. More controversial is the significance of IL-12 in autoimmune diseases.

Keywords: Cancer immunotherapy; Cytokines; Interleukins; IL-12

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Introduction

The cytokine network

The recent advances in the knowledgement of the immune system have demonstrated that the in vivo immune responses depend not only on cell-cell contact, which could explain the only local immune reactions, but also on the action of proteins provided by immunomodulating properties released by the activated immune cells, the so-called cyokines, the most important of them are represented by the group of interleukins (ILs), with more than 35 different types[1-5]. The secretion of the single cytokines influences the release of the other cytokines through feed-back mechanisms, by constituting a functional unit, the so-called cytokine network, which may be considered a system similar to the endocrine one. In any case, it has to be remarked that with respect to the endocrine system, which is mainly regulated by negative feed-back circuits, the cytokine network is founded on both negative and positive feed-back mechanisms. Then, the mechanisms involved in the control of the cytokine network are more complex than those regulating the endocrine system. The different cytokines influence both immune and inflammatory responses. Then, on the basis of their major effect on the inflammatory response, the cytokines are generally sub-divided into two main groups, represented by the pro-inflammatory and the anti-inflammatory cytokines. The main pro-inflammatory cytokines are consisting of IL-1 beta, IL-6, IL-8, IL-13, IL-17 , IL-18 and TNF-alpha[6-9], while the group of the anti-inflammatory cytokines is namely represented by IL-10[10], IL-35[11] and TGF-beta[12]. IL-2[13] and IL-12[14] are generally also included within the group of the inflammatory cytokines, but it is to be remarked that they may also exert anti-inflammatory effects in some experimental conditions, mainly consisting of the stimulation of regulatory T lymphocytes (T reg) for IL-2[15], with a consequent enhanced production of TGF-beta, and the inhibition of IL-17 secretion for IL-12[16]. Finally, cytokines have also been proven to exert endocrine, neuropsychological, metabolic and cardio-

vascular effects, by playing a fundamental role in the modulation of all biological functions[17,18], and at the other side the cytokine network is under a central neuroendocrine regulatory control[19-21]. Then, the psychospiritual status of healthy subjects and patients would influence the functionless of the immune system namely through a neuroendocrine modulation of the cytokine network. The in vivo immune responses, which depend on several cytokine interactions, as well as on the relationships among immune, nervous and endocrine systems, would represent the end-result of two opposite immune reactions, consisting of the activation or the inhibition of the immune responses, namely mediated by T helper-1 lymphocytes (TH1, CD4+CD25-) and T reg lymphocytes (CD4+CD25+), respectively. Therefore, the extreme immune alterations are consisting of an abnormally enhanced immune reaction, including that against self-antigens, and an abnormally low immune response against a different organism, which characterize the autoimmune and the neoplastic diseases, respectively. TH1 lymphocytes substantially secrete IL-2 and interferon (IFN)-gamma, TH2 cells release IL-4, IL-5, IL-6, IL-10 and IL-13, T reg lymphocytes produce TGF-beta, IL-10 and IL-35, and finally TH17 lymphocytes are the main source of IL-17. Whereas TH1 and TH2 cells are substantially stable in their functions, it would seems that TH17 lymphocytes may evolve into either T reg or TH1 cells, then in an anti-inflammatory or in a pro-inflammatory way.

The neuroendocrine control of the cytokine network

Each endocrine or neuroactive endogenous molecule may potentially influence the immune responses[19-21]. However, despite its complexity, the neuroendocrine control of the cytokine network is mainly realized by two major functional neuroendocrine units, consisting of brain opioid system-pituitary gland axis and brain cannabinergic sistem-pineal gland axis, which respectively play an inhibitory or a stimulatory effect on the immune responses. In fact, it has been shown that the opioid agents, namely the mu-agonists ones, exert an immunosuppressive activity by inhibiting IL-2 and IL-12 secretion, as well as by concomitantly stimulating that of IL-10, TGF-beta and IL-17[22,23]. On the other hand, the pineal indole hormones, namely melatonin (MLT), may stimulate both IL-2 and IL-12 secretion, respectively from TH1 lymphocytes and dendritic cells[24], while cannabinoids play their main immunomodulating effect by inhibiting IL-17 secretion[25]. The influence of stress on the immune functions would be mainly due to an enhanced brain opioid system activity, since the administration of the mu-opioid antagonist naltrexone (NTX) has appeared to abrogate the action of stress on the immune system[26]. Since the mu-opioid agonists may either stimulate IL-17 secretion, or that of IL-10 and TGF-beta[22,23], the preferential promoting effect of stress on cancer or on autoimmune disease development would depend at least in part on the preferential stimulatory effect of opioids on the secretion of IL-17, or on that of IL-10 and TGF-beta, because of the involvement of IL-17 in the pathogenesis of the autoimmune disease[27] and the immunosuppressive action of both TGF-beta and IL-10 on the anticancer immunity[10,12].

Interleukin-12

Biological properties of IL-12

IL-12 is a heterodimeric protein constituted by two sub-units, the p35 and the p40[28], and it is a part of a cytokine family, the so-called IL-12 family, which also includes IL-23, IL-27 and IL-35, all provided by specific immunoinflammatory effects. IL-12 is namely produced by the dendritic cells, the main antigen presenting cells (APC), and also by macrophages in some particular conditions. Then, IL-12 would play a fundamental role in the interactions between dendritic cells and lymphocytes for the antigen presentation. Therefore, according to these evidences, IL-12 may be considered as the main link between innate and adaptive immunity[29], the Ancient and the New Testament of the human Immunology, then it would be fundamental in the regulation among macrophages, dendritic cells, and lymphocytes. The absolute singularity of IL-12 within the cytokine group would be namely depending on the fact that it represents the main link between innate and adaptive immunity, by playing an essential role in the biological evolution of the immune system. Despite the controversial data concerning the biological effects of IL-12 reported in the literature, as well as of the most other cytokines, the immunobilogical effects of IL-12 may be summarized, as follows: 1) stimulation of TH1 differentiation (14), with a consequent enhanced production of IL-2 and interferon (IFN)-gamma; 2) stimulation of cytotoxic T lymphocyte (CD8+) activity (14); 3) inhibition of T reg cell functions, with a following diminished production of both TGF-beta and IL-10 (28); 4) inhibition of IL-17 secretion from TH-17 lymphocytes (CD4+CD17+)[29]; 5) activation of macrophages in an antitumor way[30]; 6) amplification of APC activity[31]; 7) anti-angiogenic activity[14]. Then, according to these evidences, there would no potential pro-tumoral effect for IL-12, and this is different with respect to that ofthe most other cytokines, which tend to exert both pro-tumoral and anti-tumoral immune effects, depending on the different experimental conditions. Moreover, at least in experimental studies[14,29,31], IL-12 has been proven to exert an antitumor activity superior to that of IL-2 itself, which represents the other fundamental antitumor cytokine in humans[32,33], whose anticancer action is unfortunately reduced by its potential stimulatory action not only on TH1 cells, but also on T reg lymphocytes[34], even though only in the presence of high concentrations of TGF-beta, which could be reduced by a concomitant administration of IL-12[35].

Neuroendocrine control of IL-12 secretion

In addition to the regulatory role played by the cytokine network, the secretion of IL-12, as well as that of other cytokines, is under a physiological complex neuroendocrine modulation. In more detail, IL-12 secretion is inhibited by the mu-opioid brain system and by the activation of the hyopothalamic-pituitary -adrenal (HPA) axis, since corticosteroids counteract IL-12 production and stimulated that of IL-10[22,23,28]. On the contrary, IL-12 secretion is stimulated by the pineal gland thorugh the release of MLT, which has been proven to enhance dendritic cell muturation and activation[24,33]. Since the activation of brain opioid system and HPA axis is related to stress, pain and depression[26], then to the unconscious life, whereas that of the pineal gland is connected to the perception of pleasure and to the spiritual sensitivity through its relations with the cannabinoid system of brain[25], it is possible to affirm that IL-12 secretion is stimulated by pleasure and inhibited by stress. The relation between IL-12 secretion and brain cannabinergic system is furtherly documented by the fact that IL-12 has appeared to inhibit the activity of the fatty acid amide hydrolase (FAAH), the enzyme involved

in cannabinoid degradation and metabolism, with a consequent increase in brain concentrations of the endogenous cannabinoids[25], the most important of them are the arachidonyl-ethanol-amide, also called anandamidefor its psychedelic effects, and the 2-arachidonyl-glicerol. In contrast, IL-10 has been proven to stimulate FAAH actitivity, with a following decrease in brain cannabinoid concentrations, which play a fundamental role in pleasure perception. FAAH activity isalso stimulated by leptin produced by adipocytes. Then, IL-12 would play an important neuroimmunoregulatory role in the influence of the pleasure on the immune functions, and it would represent the main cytokine involved in the stimulatory role of pleasure on the immune system. Since IL-12 stimulates the immune functions, including the anticancer immunity, whereas IL-10 exerts immunosuppressive effects, the stimulatory and the inhibitory role of IL-12 and IL-10, respectively, on brain opioid system is a further demonstration that Pleasure immunostimulates and Stress immunosuppresses.This finding is not surprising, since immune responses and pleasure perception, namely the sexual one, are the biological functions most influenced by the psychospiritual status of both healthy subjects and patients, even though the eventual immune alterations have no direct subjective effect, but only when they induce tissue damages.

Mechanisms of IL-12 antitumor activity

If cancer-related immune alterations may be synthetized as the consequence of an altered interaction among macrophages, dendritic cells and lymphocytes, then as the effect of an alteration in the the connection between innate and adaptive immunity, IL-12, at least from a theoretical physiopathological point of view, could represent the most adequate cytokine to treat cancer progression-related immune alterations. In fact, the overall main cancer-related immune cytokine anomalies, including a diminished function of TH1 lymphocytes and dendritic cells in association with a concomitant chronic inflammatory response-related enhanced activation of T reg cell and macrophage systems[36,37], could be potentially reversed and abrogated by IL-12, since IL-12 has been proven to stimulate TH1 differentiation and dendritic cellactivation, and to inhibit T reg cell generation and activity, with a following improvement in the antitumor immunity. More controversial is the significance of IL-12-induced inhibition of IL-17 secretion on the antitumor immunity[29], since IL-17 may exert both antitumoral and protumoral effects[38], respectively consisting of the inhibition of T reg cell system, and a direct stimulatory action on cancer cell proliferation, but in vivo the protumoral activity of IL-17 would be most relevant than the antitumoral one. In addition to these effects, IL-12 may act as an anticancer cytokine by also exerting an anti-angiogenic activity, with a following opposition to neo-angiogenesis-related cancer cell diffusion, and by directly stimulating the cytotoxic activity of CD8-positive T lymphocytes[14].

Il-12 and human systemic diseases

General considerations

Because of its role as a key of the evolution from the innate to the adaptive immunity, it is highly probable that IL-12 may be involved in all human systemic diseases, including autoimmunity and tumors. However, despite the fundamental role of IL-12 in regulating the whole immune system, unfortunately few clinical studies have been carried out to investigate IL-12 secretion, either alone or in asssociation with that of the other main cytokines, in the immune alteration-related human systemic diseases. In any case, it has also to be taken into consideration the fact that the simple clinical investigation of cytokine secretion does not permit to distinguish between causes and effects, since in the presence of several cytokine alterations, it becomes difficult to identify the primary cytokine alteration responsible for the other alterations. Therefore, the results of most clinical studies present the limit to put into evidence several non-specific anomalies, such as the enhanced blood levels of most inflammatory cytokines, including IL-1, IL-1 beta, IL-6 and TNF-alpha in autoimmune disease[39], and the dimished concentrations of IL-2 and IL-12 in association with high levels of TGF-beta in advanced neoplasms[40]. Then, the clinical investigation of cytokine secretion requires a well defined knowledge of the cytokine network, with the great number of interactions occurring among the different cytokines.

IL-12 in the physiopathology of cancer

The failure of an effective anticancer immune reaction in advanced cancer patients has been demonstrated to depend on several chronic inflammatory response-related events[36,37], which allow an enhanced generation and activation of T reg lymphocytes, with a consequent enhanced production of TGF-beta, one of the most potent endogenous immunosuppressive factors on the antitumor immunity because of its inhibitory effect on both IL-2 and IL-12 secretions, respectively from TH1 and dendritic cells[12]. Preliminary clinical studies have shown a progressive decline in IL-12 blood concentrations in association to that of IL-2 in cancer patients with very advanced disease[41]. If we consider that the five main advanced cancer-related immune alterations are consisting of diminished activity of TH1 lymphocytes, decreased dendritic cell function, enhanced T reg cell activity, increased activation of the macrophage system in a protumoral way, and enhanced TH17 cell function, all these alterations could be explained at least in part in terms of IL-12 deficiency, and be potentially treated by IL-12 Itself. At present, however, the possible primary role of IL-12 deficiency in determining most cancer related-cytokine alterations has still te be demonstrated. In any case, within the overall potential anticancer effects of IL-12, a particular importance has to be assigned to its modulatory action on macrophages in an antitumor way[30], since the limit of cancer immunotherapies, including that with IL-2, is at least in part represented by their possible concomitant stimulation of macrophage-mediated immunosuppressive events[32,33], which could be in any case potentially counteracted by a concomitant injection of IL-12. In fact, macrophages may mediated both protumoral and anti-tumoral effects, respectively by preferentially stimulating the release of TGF-beta or IL-12 itself. IL-12 would modulate the activity of macrophages in an antitumor way by stimulating their production of IL-12, and promoting their APC activity[30]. IL-12-induced stimulation of IL-12 production by macrophages has been shown to be inhibited by IL-11[42]. Finally, most antitumor effects of IL-12 have appeared to be mediated by IFN-gamma or at least to require its presence[43]. In fact, the stimulation of IFN-gamma secretion from TH1 cells, NK and macrophages is one of the most typical immunobiological effects of IL-12 itself.

IL-12 in the physiopathology of the autoimmune diseases

Autoimmunity-related inflammatory status would be mainly due to an enhanced production of IL-17 from TH-17 lymphocytes[44], which inhibits T reg cell activity, with a consequent possible activation of autoreactive T cells. IL-12 seems to be also involved in the pathogenesis of the autoimmune diseases, particularly in those preferentially mediated by TH1 lymphocytes[45,46], including multiple sclerosis. However, if it is true that IL-12 may negatively influence the clinical course of the autoimmune diseases by determining a further decline in the already low T reg cell activity, it is also true that it may inhibit IL-17 secretion, which plays an important role in the induction and evolution of the autoimmune processes. Therefore, the end-result of IL-12 in the autoimmunity could depend on the balance between its potential negative and positive effects. Then, a clinical evaluation of the ratio between IL-17 and IL-12 blood concentrations could constitute a good index to monitor the immune status and the clincal evolution of patients affected by autoimmunity.

Therapeutic Implications Of Il-12

1IL-12 in cancer immunotherapy

The fundamental role of IL-12 in the immunotherapy of cancer is justified by its multiple anticancer effects, including stimulation of TH1 and dendritic cell functions, and inhibition of T reg cell- and macrophage-mediated immunosuppressive events. In fact, in experimental studies IL-12 has been proven to play an anticancer activity superior to that of IL-2[47]. Unfortunately, very few clinical studies have been carried out up to now with IL-12 in cancer immunotherapy[47]. IL-12 alone has appeared to have low anticancer efficacy, generally limited to malignant melanoma and renal cell cancer, which represent the classical neoplasms more sensitive to an immunotherapeutic approach. IL-12 alone has been shown to induce lymphocytopenia[14,43]. Then, on the basis of the fundamental role of lymphocytes in tumor cell destruction, the low in vivo anticancer activity of IL-12 might depend at least in part on its lymphocytopenic effect. On the contrary, preliminary clinical studies in cancer patients have shown that the concomitant administration of IL-2 not only abolish IL-12-induced lymphocytopenia, but may paradoxically determine a lymphocyte increase superior to that achieved by IL-2 alone[48]. Therefore, the association between IL-12 and IL-2 would constitute at present the more suitable cytokine combination in cancer immunotherapy of human neoplasms, at least from a physiopathological point of view.In fact, IL-2 and IL-12 association is potentially able to activate both antigen-dependent and antigen-independent anticancer cytotoxicity, namely mediated respectively by cytotoxic T lymphocytes, mainly activated by IL-12, and NK cells after their stimulation by IL-2 and evolution into lymphokine-activated killer cells (LAK), characterized by changes in their own morphology. NK cells would exert cytotoxic antitumor activity only against artificial laboratory cancer celllines, whereas LAK cells are able to destroy fresh cancer cells drawn from the tumor of patients[32]. The fundamental role of IL-12 and IL-2 in human cancer immunotherapy is furtherly shown by the fact that it has been proven that cancer patients responsive to IL-2 immunotherapy are generally those, who endogenously produce IL-12 in response to IL-2 injection[33]. Finally, the importance of IL-2 and IL-12 in cancer immunotherapy is also documented by the fact that within the cytokine network, IL-2 and IL-12 probably represent the only cytokines, which are able to enhance their production by themselves, IL-12 by stimulating IL-12 secretion from macrophages instead of IL-10, and IL-2 by inducing the proliferation of the same IL-2 producing TH1 lymphocytes because of their concomitant expression of IL-2 receptors and their consequent clonal expansion.

IL-12 in autoimmunity and allergy

Both IL-2 and IL-12 could potentially exacerbate the clinical course of the autoimmune diseases, as demonstrated by the fact that the development of an autoimmune disease may represent one of the most common complications of cancer immunotherapies, either with cytokines, or anti-check point inhibitor monoclonal antibodies. However, on the same way both IL-2 and IL-12 could paradoxically exert some benefits also in the autoimmune processes, IL-2 by stimulating T reg cell activation, and IL-12 by inhibiting IL-17 secretion. Obviously, it is probable that the preferential positive and negative effects of both IL-12 and IL-2 in the autoimmune diseases may depend on the different doses and schedule of administration. In more detail, IL-12- induced inhibition of T reg cell system, which would allow a further exacerbation of the autoimmune dynamics, could be counteract by a concomitant IL-2 injection, because of its potential stimulatory activity on T reg cells. As far as the allergy is concerned, IFN-gamma deficiency, which characterizes the allergic diseases, would depend on a defect of IL-12, which plays a fundamental role in the stimulation of IFN-gamma production[21]. In any case, it hasto be taken into consideration that in addition to a direct injection of the different cytokines, another way to act on the cytokine network may be represented by the administration of natural agents provided by well demonstrated and specific effects on cytokine secretions. Within the molecules drawn from plants, one of the main natural agent provided by stimulatory action on IL-12 production is berberine[49], which is contained in Berberis vulgaris and in various other common plants. Because of its stimulatory role on IL-12 secretion, berberine may induce a shift from a TH2 to a TH1 immune responses. Berberine ha salso been proven to exert anti-atherosclerotic and anti-arythmic effects, and to prevent the cardiac hypertrophy[50]. The pineal hormone MLT has also appeared to promote dendritic cell functions and IL-12 production[24].

Conclusion

Every human systemic disease-related immune alteration may be synthetically considered as the expression of an altered connection between innate and adaptive immunity. Then, by representing one of the main factors involved in the interaction between old and new immunity, IL-12 could play a potential fundamental role in the treatment of all human systemic diseases. The antitumor properties of IL-12 have been confirmed also in humans, even though only in a preliminary manner, and at present the maximal anticancer efficacy of IL-12 has been seen in association with IL-2 (48). On the contrary, its possibile negative or positive therapeutic effect in the treatment of the autoimmune diseases has still to be better investigated and defined.

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