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A Rediscovered Immunomodulatory Action of Cancer Chemotherapy to Pilot the Cytokine Network in an Antitumor Way

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SUMMARY

The recent proposal of cancer immunotherapy with anti-checkpoint inhibitor monoclonal antibodies, as well as the previous immunotherapy with IL-2, would require an interpretation of cancer chemotherapy not only in terms of therapeutic strategy carried out to destroy cancer cells, but also as an approach potentially able to influence and modulate the cytokine network in an attempt to correct cancer-related cytokine alterations responsible for tumor progression itself. This statement is justified by the fact that the efficacy of cancer immunotherapy is depending at least in part on the cytokine secretions of patients. Despite the great number of cancer-related cytokine alterations, the main alterations provided by a physiopathological and prognostic significance would consisting of low blood levels of IL-2 and IL-12 in association with abnormally high concentrations of IL-1, IL-6, TNF-alpha, IL-10 and TGF-beta. The effects of chemotherapy on cytokine secretions have appeared to depend on the type of agent, as well as on its dosage and schedule of administrarion. At present, the main effects of chemotherapy provided by a potential clinical application would be represented by the stimulatory action of adriamycin on IL-2 secretion, the inhibitory effect of cisplatin on IL-6 secretion, the stimulatory effect of gemcitabine on IL-12 production, and the inhibitory action of both cyclophosphamide and gemcitabine on regulatory T cell system. Then, the future chemo-immunotherapeuticregimenswouldhavenot to be elaborated not only on the bases of empiristic criteria and on the cytotoxic effects of che various chemotherapeutic agents, but also by taking into consideration their specific activity on the secretion of those cytokines, whose anomalous production has to be corrected and neutralized.

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Introduction

Cancer chemotherapy (CT) and immunotherapy (IT) are commonly considered as two different and opposite strategies in the treatment of human neoplasms. In fact, CT mainly act by exerting a direct cytotoxic effect against cancer cells, whose efficacy is at least in part counteracted by its inhibitory action on the immune functionless, whereas IT may control tumor growth by generating an effective antitumor immune response. On the contrary, several experimental studies have demonstrated that the immune effects of CT may change in relation to the type of immune response, and this finding would depend on the different sensitivity of the various immune cells to the cytotoxic action of CT [1,2]. Then,if it is true that cancer CT tends to constantly inhibit the anti-viral and the anti-bacterial immunities, it is also true that CT may exert both suppressive and stimulatory effects on the antitumor immunity, whose physiology is different from that of the other forms of immune responses [3].

This evidence is not surprising, if we consider that the antitumor immunity would represent the end result of opposite stimulatory and suppressive mechanisms, which are mediated by different immune cells, provided by a different sensititivity to CT cytotoxicity. Therefore, a possible association between CT and IT in an attempt to amplify the efficacy of IT itself,obvioulsy requires a well define knowledge of the mechanisms involved in the generation and in the suppression of an effective anticancer immune response. From this point of view, the recent advances in the knowledge of the immune functionless have demonstrated that the in vivo activities of the different immune cells depend not only on their functional status, but also on a central regulation exerted by the cytokine network, represented by the systemic production of a great number of proteins by the activated immune cells, the so-called cytokines or interleukins, which are provided not only by immunomodulating activity, but also by systemic effects on endocrine, nervous and cardiovascular systems [4,5].

Then, the immune system would exert a systemic regulation on the whole human biology through the secretion of several cytokines, which are the mediators of the systemic effects of the immune reactions. Moreover, it has been shown that the immune responses represent only a part of a more general biological response, which constantly includes a concomit antinflammatory and angiogenic response [6-8]. Therefore, the elaboration of possible chemo-immunotherapeutic antitumor strategies would primarily requires the investigation of CT effects on the secretion of the different cytokines, or at least its effects on the main cytokines involved in the biological immunoinflammatory response, including IL-6, IL-10, IL-2, IL-12, TGF-beta and TNF-alpha [9-14]. The immune effects of cancer CT on cytokine secretions depend on both dose

and type of chemotherapeutic agent (1,2).Finally, low-dose CT has been proven to exert anti-angiogeniceffects [15].

Classification of Cytokines

According to their major biological function, cytokines are generally sub-divided into two main classes, consisting of inflammatory and anti-inflammatory cytokines. The main pro-inflammatory cytokines are IL-6, IL-1 beta, TNF-alpha, IL-17, IL-2, and IL-12, whereas the most important anti-inflammatory cytokines are substantially represented by the only TGF-beta and IL-10 [13,10]. Another possible classification may be based on the main immune cell responsible for the secretion of each specific cytokine, consisting of monocyte-macrophage, dendritic cell or lymphocyte origins. IL-2 and IL-12 are namely secreted by TH1 cells and dendritic cells, respectively, event though IL-12 may be produced by some macrophage subsets [11,12]. IL-6, IL-1 beta and TNF-alpha may be mainly produced by macrophages, while TGF-beta ad IL-10 may be secreted by both monocyte-macrophage system, and regulatory T lymphocytes (T reg) (CD4+CD25+) [9,14,16].

Finally, cytokines may be classified according to their effects on the antitumor immunity, and on the basis of the great number of experimental and clinical studies available up to now, the only cytokines provided by a clear anticancer activity in humans are IL-2 and IL-12, even though a potential antitumor activity may be exerted also by IL-7 and IL-15 [11,12,17]. Most other cytokines, including both inflammatory cytokines, such as IL-6, TNF-alpha, and IL-17, and anti-inflammatory cytokines, such as TGF-beta and IL-10, would play a major pro-tumoral activity, due to different mechanisms, namely consisting of inflammation-related immunosuppression of the antitumor immunity for the inflammatory cytokines and of a direct inhibition of the main cells involved in the antitumor immunity, represented by T helper-1 (TH1) (CD4+CD25-CD17-), cytotoxic T lymphocytes (CD8+) and dendritic cells for the anti-inflammatory cytokines TGF-beta and IL-10. IL-17 has also been proven to promote tumor growth by stimulating cancer cell proliferation [13,10,7,18].

However, it has to be remarked that within the cytokine group, IL-2 and IL-12 may exert both inflammatory and anti-inflammatory actions [3]. In fact, IL-2 may play inflammatory effects by activating the whole immune system, including the macrophage one, and anti-inflammatory ones through an inhibition of IL-17secretion and a stimulation of T reg cell generation and this evidence would explain the potential therapeutic role of IL-2 in both tumors and autoimmune diseases [19]. On the same way, IL-12 may play either inflammatory effects by promoting TH1 differentiation, cytotoxic T cell activation and inhibition of T reg cell functions or anti-inflammatory actions by inhibiting IL-17 secretion from TH17 lymphocytes (CD4+CD17+), which would represents the main cells responsible for the induction of autoimmune reactions by inhibiting T reg cell generation and activation [12,20,21,18].

The Dynamics of the Anticancer Immunity

The discovery of the fundamental role played by the cytokines in the control of the immune functionless has to allow to reinterpretate the anticancer immunity in terms of endogenous functional status of the cytokine network. Despite its complexity and the great number of controversial results reported in the literature, the antitumor immunity in humans has been proven to be substantially the final result among algebric effects, resulting from a conflict between immunostimulatory and immunosuppressive activities. The antitumor immunity is fundamentallyconsisting of the generation of both antigen-independent and antigen-dependent anticancer cytotoxicity, which are respectively mediated by NK cells after their evolution into LAK cells induce by IL-2 , and by cytotoxic T lymphocytes under their activation induced by IL-12 [11,12]. On the other hand, the anticancer immunity is suppressed by two main cell systems, consisisting of macrophage and T reg cell systems [7,16].

In more detail, macrophages would mainly inhibit NK cell-mediated cytotoxicity by blocking LAK cell activation through the release of IL-6, while T reg lymphocytes may directly inhibit the T cell-mediated specific cytotoxicity, namely through the release of TGF-beta, which represent the main immunosuppressive endogenous factor on the anticancer immunity [11,15,13]. Moreover, it has been shown that macrophages, in addiction to their possible inflammation-mediated direct suppression of the anticancer immunity, may counteract the anticancer immunity by promoting the generation of T reg cells, which would mainly depend on the production of TGF-beta itself and IL-10 by myeloid precursor suppressor cells (MPSC) [7,22]. Then, the inihibition of the anticancer immunity would be namely due to the action of T reg cells [16].

Therefore, the modulation of the immuno-biological response in an attempt to allow an increased efficacy of cancer IT would primarily consist of a control of T reg cell generation and activation. In fact, it has been shown in experimental conditions that removal of T regs results in the enhancement of the efficacy of the antitumor immune response [23,24]. Moreover, dendritic cell differentiation and maturation have appeared to be blocked by VEGF [25]. Then, VEGF may promote tumor progression through both angiogenic and immunosuppressive effects. By synthetizing, at least from a theoretical point of view, the optimal cytokine condition for the generation of an effective anticancer immune response would consist of an increased secretion of IL-2 and Il-12 in association with a diminished production of IL-6, TNF-alpha, IL-10 and TGF-beta. On the contrary, the evidence of low levels of IL-2 and IL-12 in association with high concentrations of TGF-beta, IL-6, IL-10 and TNF-alpha, has appeared to be associated with a poor prognosis and a lower survival in cancer patients [3,26]. The occurrence of high levels of TNF-alpha would be responsible for the neoplastic cachexia [14].

The Physiology of Regulatory T Lymphocytes

The biological significance of T reg cells is consisting of the protection against an excessive inflammatory respon se and the development of autoimmune reactions against self-antigens. On the contrary, because of their suppressive activity on the anticancer immunity, an enhanced T reg cell activation the role of T reg cells is negative in the presence of neoplasms. Then, since the main cells responsible for the inhibition of the antitumor immunity are represented by T reg lymphocytes namely through the release of TGF-beta, the most important immunosuppressive endogenous factor the IT of cancer would have, substantiallyconsist of a block of T reg cell activity [13,23,24].

T reg cells may block the anticancer immunity through several mechanisms, which however have been substantially well clarified, and they are due to either a direct secretion of immunosuippressive molecules, namely TGF-beta itself, or a cell-cell contact after the expression on cell surface of particular molecules, the so-called checkpoint inhibitors, namely PD-1 with its twoligands, PD-L1 and PD-L2, and CTLA-4 [16, 27,28]. Their block through the administration of specific monoclonal antibodies (MABs) has appeared to allow an effective anticancer immune reaction and a control of the neoplastic growth. Since the occurence of

autoimmune reactions under the artificial cancer IT with MABs may predict the efficacy of treatment, this evidence would furtherly confirm that tumor control achieved by cancerIT has to require the inibibition of T reg cell activity.

In any case, it has to be remarked that T reg cell activation with a following enhanced TGF-beta secretionis under both cytokine and neuroendocrine regulation. In more detail, from a neuroendocrine point of view, T reg cells are stimulated by corticosteoids, mu-opioid agonists, beta-adrenergic agonists and vitamin D3 whereas they are inhibited by beta-adrenergic antagonists and mu-opioid antagonists, such as naltrexone (NTX) [29,30,31,32,33]. The stimulation of T reg cell functions would represent the main mechanism responsible for the anti-inflammatory and immunosuppressive effects of cortisol itself. Moreover, it is interesting to observe hat both mu-opioidagonists and beta-adrenergic agents may exert selective actions on the different T lymphocyte subsets, by stimulating the only T reg cells and inhibiting proliferation and function of the other T cell subpopolations, namely TH1 cells themselves, as well as the dendritic cells.

Finally, as far as the action of the pineal hormone melatonin (MLT), whose immunomodulating properties are well known the results are still controversial, and at present it seems that it may either inhibit or stimulate T reg cell system, depending on itsfunctional status, and thatin the presence of an abnormallyenhanced T reg cellfunction, aswellas like thatoccurring in the neoplasticdiseases, MLT wouldexert an inhibitoryeffect on T reg cells and on TGF-beta secretion [34,35]. On the other side, from a cytokine point of view, T reg cells are stimulated by TGF-beta and IL-10 themselves, as well as by IL-2, whereas they are inhibited by IL-12 and IL-17 [16,19,20,36]. The regulation of T reg cell system is illustrated in Figure 1.

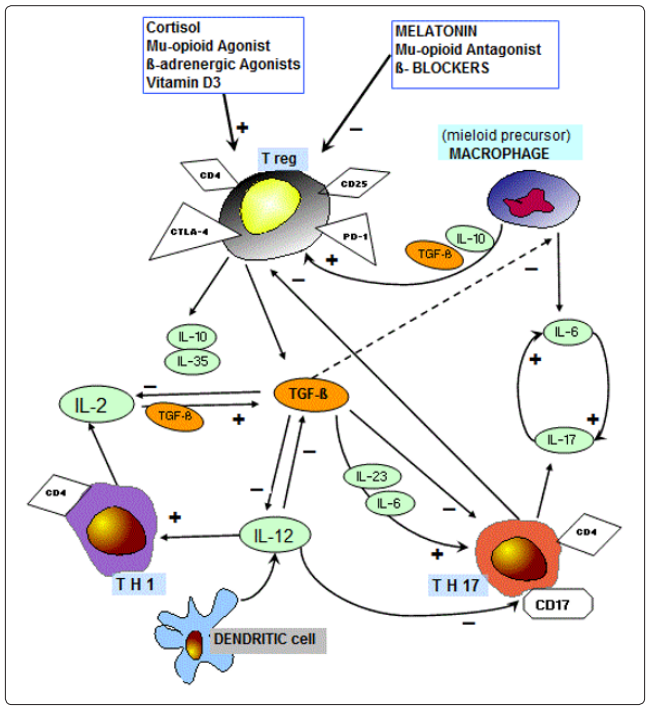

Figure 1: The regulation of T reg cell system

Immune Effects of Cancer Chemotherapy on the Cytokine Network
It is known that CT is more effective against rapidly proliferating cells, whereas its cytotoxic activity is low against less proliferating cells. Then, the efficacy of CT in solid tumors, whose proliferation rate is generally lower than that shown by the normal cells, would be due to effects other than the only cytotoxic action, and they include a modulatory activity on both immune and angiogenic processes, which are under a cytokine regulation. Therefore, to explain the immunomodulatory effects of cancer CT, we have primarily to investigate its influence on the cytokine network, since both anticancer immune response and angiogenic processes would depend on its functional status.

At present, it is known that the immune effects of CT on the antitumor immunity depend on the different chemotherapeutic agents, their dosage and schedule of administration, but in any case,it would have to be constantly taken into consideration that the effects of the single chemotherapeutic agent may be different in vivo and in vitro, and may vary in relation to the different experimental conditions, as well as in association with other chemotherapeutic drugs [1,2]. However, despite the controversial results, some define conclusions may be drawn, at least for the most commonly used chemotherapeutic agents, including anthracyclines, cisplatin (CDDP) and its analogues, cyclophosphamide (CTX), 5-fluouracil (5-FU) and its analogues, gemcitabine (GEM), and irinotecan (CPT-11) [1,2,37].

In more detail, low-dose adriamycin (ADM) may stimulate TH1 lymphocytes, with a following enhanced production of IL-2 and gamma-IFN [38]. CDDP has appeared to inhibit both IL-6 and IL-2 secretions [39]. 5-FU may reduce the secretion of IL-10 and IL-2 (37). In addition to an inhibition of IL-10 and IL-2 secretions, CPT-11 may counteract also TNF-alpha release [37]. Low-dose CTX has appeared to selectively reduce T reg cell count [40]. Finally and interestingly, GEM may inhibit T reg cell generation by blocking the action of MPSCs without affecting IL-2 secretion and modulate dendritic cell activity with a stimulation of IL-12 secretion, which has been proven to either directly stimulate T cell-mediated antigen-dependent cytotoxicity or pilot the macrophage function in an antitumor cytotoxic way [12,41,42,43]. In contrast, IL-4 secretion does not seem to be influenced by cancer CT [37].

Melphalan has also appeared to play interesting immune effects, consisting of stimulation of TH1 and inibibition of TH2 lymphocytes [44]. Most negative immune effects of cancer CT, namely the reduced secretion of IL-2, may be corrected by the simple concomitant administration of high-dose pineal indole MLT (34), as confirmed by preliminary clinical studies [45]. Finally, it has been shown that the efficacy of CT may be enhanced by the concomitant administration of anti-oxidant agents [46]. Since MLT would represent one of the most potent natural anti-oxidant agent this finding would be already sufficient to explain MLT-induced enhanced efficacy of cancer CT. Then, according to the knowledgements available up to now, GEM, low-dose ADM and low-dose CTX would seem to represent the most effective CT regimen to improve the antitumor immunity in cancer patients [47].

Unfortunately, few data only are available about the effects of CT on the secretion and activity of TGF-beta, the main endogenous immunosuppressive factor, which has also appeared to be responsible at least in part for the resistance to CT in several tumor histotypes, including the triple-negative breast cancer [48,49]. Paclitaxel has been shown to promote TGF-beta production, and this finding would be responsible to the resistance to paclitaxel itself, since the administration of anti TGF-beta type 1 receptor kinase MABs, EW-7197 or LY2157299, may abrogate the chemoresistance [48,49].

The Clinical Evaluation of the Immune Effects of Chemotherapy
Obviously, the clinical management of the immune evaluation

of cancer patients has to be different and more simple than that performed in experimental conditions. From this point of view, recent clinical investigations have shown that the complexity of cytokine interactions involved in the anticancer immunity or in its opposition, may allow as the end-result a well defined lymphocyte-to-monocyte ratio (LMR) since a decline in LMR value, due to either lymphocyte count decline, or monocyte number increase, has been proven to be associated with an increase in T reg cell count by reflecting a suppressive status of the anticancer immunity. In fact, the evidence of an abnormally low LMR has appeared to predict a negative prognosis and a lower survival in most tumor histotypes [50,51,52].

The effect of CT on LMR may be different in relation to each cancer patient, since LMR has appeared to either decrease, or increase under CT, and CT-induced increase in LMR values just after the first chemotherapeutic cycle has been proven to predict the efficacy of CT itself (50), by confirming that CT may not only inhibit, but also improve the antitumor immunity in cancer patients, because of the different effect of CT on the various immune cell subsets. As far as the profile of LMR in cancer patients is concerned, generally the increase in monocyte count preceedes the decline in lymphocyte number [50].

The increase in monocyte count is associated with an increase in IL-6 blood concentrations, whereas lymphocyte number decline is depending on a decline in those of IL-2, and the evidence of abnormally low levels of IL-2 and of abnormally high IL-6 levels has been proven to be associated with a lower survival in advanced cancer patients. Finally, the occurrence of high levels of TGF-beta has appeared to be also associated with a less favourable prognosis in human neoplasms [13,16]. Therefore, from a clinical point of view, the evaluation of LMR prior to therapy and after each single cycle of CT, in association with an eventual measurement of IL-2, IL-6 and TGF-beta blood concentrations, may be considered as sufficient to investigate the immune status of cancer patients in basal conditions and under cancer CT.

Conclusions

At present, few studies only combining IT and CT have been performed in the treatment of human neoplasms, namely because it has been widely assumed that CT may be only immunosuppressive on the anticancer immunity. Then,it was assumed that CT would counteract IT-induced stimulation of the antitumor immunity, and the only benefit of the association between CT and IT was belived to be represented by the potential enhancement of tumor immunogenicity following the toxic action of CT against cancer cells. Moreover, CT-induced reduction of tumor mass may also enhance the action of IT, whose efficacy has been proven to be greater in the presence of a minimal residual disease. Then, the debulky action of CT could per se improve the efficacy of IT.

On the contrary, recent experimental and clinical studies have demonstrated that CT may modulate cytokine secretions, and upregulate cell surface expression of major histocompatibility complex molecules, which are fundamental in regulating immune cell-mediated tumor cell destruction [1,2,37]. Then, since the antitumor immunity depends on complex interactions among several cytokines, and the efficacy of cancer IT would depend not only on tumor biological characteristics, but also on the immune status prior to therapy of cancer patients, CT could be successfully associated with IT to modulate and correct those cytokine alterations, which negatively influence the efficacy of IT itself, namely the evidence of low levels of IL-2, and high TGF-beta and IL-6 concentrations [50].

Therefore, the future chemoimmunotherapeutic combinations would have to be elaborated not only on the basis of the type of CT most effective against some tumor histotypes, but also on the basis of CT effects on the cytokine network in an attempt to normalize the type of cytokine alteration occurring in the single cancer patient. Most immunochemotherapeutic combinations proposed up to now have used IL-2 as immunotherapeutic agent, either alone or in combinations with some chemotherapeutic agents, including 5-FU, GEM and CDDP, with, however, controversial results because of the too empiristic criteria of their combinations [53]. In any case, at present, however, low-dose ADM, low-dose CTX and GEM would represent the chemotherapeutic regimens more provided by potential stimulatory effects on antitumor immunity.

The elaboration of possible association between CT and the more recent cancer immunotherapies with specific MABs against checkpoint inhibitor molecules, namely PD-1 and CTLA-4, which are responsible for the activation of T reg cells with a following suppression of the antitumor immune response, would have to be founded not only on the cytotoxic properties of CT against each specific tumor histotype, but also on the immune status of patients, namely their endogenous cytokine secretion, in an attempt to correct eventual alterations through the administration of chemotherapeutic agents provided by some specific effects on the cytokine network. In fact, it has been shown that the efficacy of cancer IT with anti-PD1 and anti-CTLA-4 MABs is depending on the biological response of patients, and that it is higher in the presence of an increase in LMR values under treatment, due to either lymphocyte increase, or monocyte decline, whereas no efficacy may be expected in the presence of an evident lymphocyte decline on therapy [27,28,50].

At present, the only immune molecule able to induce a clear lymphocytosis still remains IL-2 alone [11,53). Therefore, low-dose IL-2 could successfully associated with anti-check point inhibitors in an attempt to induce an increase in lymphocyte count, as already observed in experimental conditions [54]. The immunotherapeutic approach to cancer cure, finally elaborated on the basis of a well defined knowledge of the immune physiopathology and cytokine network, could allow in a next future to really win the human neoplastic disease by simply restablishing the neuro-endocrino-immune status of health.

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