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Vaccines & Antibodies

Potential use of IL-2/anti-IL-2 antibody immune complexes for the treatment of cancer and autoimmune disease

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Initially discovered as a potent T cell proliferation factor, IL-2 was soon used for cancer immunotherapy, especially for metastatic melanoma and renal cell carcinoma; however, the severe side effects of IL-2 therapy, plus the negative role of IL-2 in maintaining of $CD4^+$ $CD25^+$ T regulatory cells (Tregs), has somewhat dampened enthusiasm for using IL-2 in immunotherapy. This opinion article discusses the possibility of combining IL-2 with certain anti-IL-2 antibodies for reducing the dose of IL-2 needed and preferentially stimulating effector T cells, but not Tregs, an approach that might provide an improved strategy for anticancer immunotherapy. Alternatively, complexes of IL-2 with other anti-IL-2 antibodies can selectively stimulate Tregs and could, therefore, be useful for treating autoimmune diseases.

Keywords: activation-induced cell death, autoimmune disease, autoimmunity, cancer, cytokine-binding protein(s), IL-2, immune complexes, immune modulation, memory T cell, T regulatory cell

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1. Introduction

IL-2 belongs to the family of common gamma chain (γ_c) cytokines, which comprises a group of cytokines that use the γ_c receptor and play a central role in lymphocyte generation, survival and homeostasis. Besides IL-2, also IL-4, -7, -9, -15 and -21 belong to this cytokine family [1]. Despite common usage of this shared γ_c subunit, individual γ_c cytokines are synthesised by different cells. IL-2 is produced mainly by activated $CD4^+$ T helper cells [2-4], although $CD8^+$ T cells, natural killer (NK) cells, NK T cells and dendritic cells activated by microbial stimuli have also been reported to produce IL-2 in low levels [4,5]. IL-2 mediates its action in an autocrine or paracrine fashion by binding to the IL-2 receptor (IL-2R), which typically consists of three receptor subunits called IL-2Rα (CD25), IL-2Rβ (CD122) and γ_c (**Figure 1**) [2,3]. It is thought that *in vivo* responses to IL-2 are mediated through its interaction with the high-affinity trimeric IL-2R, comprised of all three receptor subunits. Conversely, CD122 and γ_c by themselves can form a dimeric low-affinity IL-2R, which binds IL-2 with ~ 100-fold lower affinity, but which nevertheless transmits signals following association with IL-2 [6]. Hence, CD25 is not essential for IL-2 signalling, but instead confers high-affinity binding of IL-2 to its receptor, whereas CD122 and γ_c are crucial for signal transduction as the absence of either subunit leads to the abrogation of IL-2 signals to the cell.

IL-2 was initially discovered because of its potent *in vitro* T cell growth function [7,8]. Once cloned and available in purified form, recombinant IL-2 was used *in vivo* in the immunotherapy of cancer [9]. This approach showed great promise, as large metastatic tumours could be treated effectively in mice, although

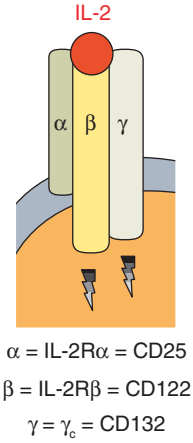

Figure 1. IL-2 receptor. The IL-2R typically consists of three receptor subunits called IL-2Rα (CD25), IL-2Rβ (CD122) and γ_c (CD132). CD25 is not essential for IL-2 signalling, but instead confers high-affinity binding of IL-2 to its receptor, whereas CD122 and γ_c are crucial for signal transduction. CD25, CD122 and γ_c comprise the high-affinity trimeric IL-2R. Conversely, CD122 and γ_c alone can form a dimeric low-affinity IL-2R, which binds IL-2 with ~ 100-fold lower affinity, but which nevertheless transmits signals following association with IL-2.
IL-2R: IL-2 receptor.

at the cost of significant IL-2-induced toxicity. With the generation of IL-2- ($IL\text{-}2^{-/-}$) and IL-2R-deficient ($CD25^{-/-}$ and $CD122^{-/-}$) mice, which all suffer from uncontrolled immune activation causing autoimmune haemolytic anaemia and inflammatory bowel disease, it became evident, however, that IL-2 also governs important roles in maintaining peripheral immune tolerance [10-12]. Furthermore, IL-2 did not seem to be essential for T cell proliferation *in vivo*, as these mice contained above normal T cell numbers. It was then shown that a population of so-called $CD4^+$ $CD25^+$ T regulatory cells (Tregs) were missing in these mice, and the transfer of these cells from normal wild-type mice to $IL\text{-}2R^{-/-}$ animals rescued the latter from autoimmunity and inflammation. Thus, it was proposed that IL-2 signals were essential for the maintenance of functional Tregs, which are able to inhibit or dampen overt T cell responses to self-antigens, including tumour antigens [13-17].

Although a role for IL-2 in protective T cell immunity has been recently rediscovered [18-20], researchers are questioning the use of IL-2 for antitumour immunotherapy [21]. This review discusses these issues and also describes how combining IL-2 with certain anti-IL-2 monoclonal antibodies (mAb) can preferentially stimulate cytotoxic $CD8^+$ T cells and, thereby improve IL-2-mediated anticancer immunotherapy [22]. By contrast, combinations of IL-2 with other anti-IL-2 mAbs leads to selective stimulation of Tregs, which might open new treatment options for autoimmune diseases.

2. IL-2-based immunotherapy

The original observation that certain tumours evoke an effective antitumour immune response in mice, mediated predominantly by T cells, opened new ways to explore immunotherapeutic approaches for the treatment of cancer. The subsequent discovery of IL-2 as a potent T cell growth factor *in vitro* was soon translated into immunotherapeutic anticancer strategies. In the course of these studies, recombinant IL-2 was shown to have potent antitumour activities in several tumour models in mice. Based on these data, IL-2 treatment was tested in metastatic cancers in humans, and promising results were obtained for renal cancer and melanoma. Subsequently, the FDA approved high-dose (HD) bolus IL-2 treatment in 1992 and 1998 for the treatment of metastatic renal cell carcinoma and metastatic melanoma, respectively. HD IL-2 treatment consists of cycles of 15-minute intravenous infusions of 600,000 – 720,000 IU/kg of recombinant human (rh)IL-2, every 8 h for up to 14 doses. After a 5- to 9-day rest period, an additional 14 doses of HD rhIL-2 are given, followed by a 6- to 12-week interval. Patients with objective tumour regression or stable disease receive further courses of treatment up to a maximum of five treatment courses [23,24]. Following this strategy, selected patients with metastatic melanoma and renal cancer were enrolled in several studies, which resulted in an overall objective response rate to therapy of 17%. Complete regression of tumours was seen in 6.6 and 9.3%, and partial regression occurred in 8.2 and 9.7% of patients with metastatic melanoma and metastatic renal cancer, respectively. It was then concluded that the treatment with HD bolus IL-2 led to a durable and complete regression of disease in ~ 8% of patients with metastatic melanoma or renal cancer, and, thus, should be considered for the initial treatment of selected patients with these malignancies.

3. Drawbacks of IL-2 treatment

3.1 IL-2 treatment-induced toxicity

In preclinical mouse studies, and subsequently in clinical trials, significant toxicity caused by the administration of HD IL-2 was noted. Thus, patients receiving HD IL-2 treatment frequently experience adverse effects, including cardiovascular, pulmonary, renal, hepatic, gastrointestinal, neurological, cutaneous, haematological and systemic events (**Table 1**). These side effects are quite severe, resembling the clinical manifestations of septic shock, and require intensive monitoring and in-patient management [25,26]. Most toxic side effects disappear within a couple of days after cessation of HD IL-2 treatment.

The majority of these adverse events can be explained by the development of so-called vascular (or capillary) leak syndrome (VLS). Caused by pathologically increased vascular permeability, VLS leads to egress of intravascular fluid. Following this is intravascular volume depletion, causing a

drop in blood pressure and a compensatory increase in heart rate. The fluid extravasation occurs in multiple organs, leading to interstitial pulmonary oedema, pleural effusions, ascites and cutaneous oedema, among others. Perturbation of blood flow with formation of thrombi in the microvasculature and reduced blood pressure can then lead to hypoxia of vital organs, such as the heart, central nervous system, lungs, kidneys, liver and gastrointestinal system [27,28].

Similar to patients receiving HD IL-2 treatment, mice given 2×10^6 IU/kg of rhIL-2 twice daily for 4 days experience significant IL-2-mediated toxicity, including VLS [29-31]. Both in mice and humans, VLS correlates directly with the duration and dose of IL-2 treatment. Thus, a low-dose regimen using 72,000 IU/kg of rhIL-2 caused significantly less severe side effects in humans; however, the response rate with low-dose IL-2 was inferior to HD IL-2 treatment, and this is why low-dose IL-2 was not recommended for the treatment of metastatic cancers [32,33]. As VLS is the complication causing most of the morbidity, research has focused on identifying the factor(s) leading to this breach of vascular integrity. Theoretically, two possibilities could account for VLS: first, IL-2 itself could directly influence endothelial cells and lead to increased vascular permeability; alternatively, IL-2 could affect the integrity of blood vessels indirectly, for example, by stimulating T cells to produce vasoactive mediators.

Experiments in which mice were preconditioned by irradiation or immunosuppression showed a marked reduction in IL-2-mediated VLS, suggesting the involvement of radiosensitive cells [29,34]. Subsequently, it was demonstrated that depletion of NK cells by an antibody to asialo-GM1 or to NK1.1 markedly reduced VLS without much compromising the therapeutic antitumour effect of IL-2 [30,34,35]. By contrast, depletion of T cells resulted in considerable reduction of the therapeutic antitumour effect without reducing toxicity. Further evidence for an involvement of NK cells in VLS came from studies using IL-15-deficient mice, which show a severe reduction in NK cell numbers. In comparison with wild-type IL-15-proficient animals, these mice exhibited considerably reduced pulmonary VLS when treated with IL-2 [36]. Furthermore, administration of antibodies against TNF-α or pentoxifylline, an inhibitor of TNF-α, was able to reduce IL-2 toxicity without compromising the antitumour effect [37,38]. Although TNF-α might be produced by a series of cell types, IL-2 treatment might preferentially lead to TNF-α production by IL-2-responsive NK cells. Therefore, the implication is that VLS is caused by NK cells, whereas the therapeutic effects of IL-2 depend on T cells. However, there are also reports that IL-2-induced VLS is due in part to a direct action of IL-2 on endothelial cells [39,40].

Table 1. Frequently observed adverse events during high-dose IL-2 treatment. Modified from [24,32,72].

Adverse event	Incidence (%)
Cardiovascular	
Hypotension	64
Tachycardia	17
Myocardial ischaemia and infarction	4
Pulmonary	
Dyspnoea	31
Adult respiratory distress syndrome (ARDS), pulmonary oedema	16
Renal	
Oliguria	49
Increased creatinin levels	35
Hepatic	
Elevated bilirubin levels	51
Elevated transaminase levels	39
Elevated alkaline phosphatase levels	13
Gastrointestinal	
Nausea, vomiting	55
Diarrhea	54
Stomatitis	14
Neurological	
Depression, mental confusion	30
Somnolence (very seldom coma)	17
Cutaneous	
Skin rash	27
Exfoliative dermatitis	15
Haematological	
Thrombocytopenia	43
Anaemia	29
Leukopenia	21
Systemic	
Weight gain > 5% of baseline	50 – 60
Fever, chills	47
Malaise	34
Infection*	15

* Infections could be controlled when antibiotic prophylaxis became routine for high-dose IL-2 treatment.

3.2 Activation-induced cell death

Apart from toxicity, there are two concerns with regard to IL-2 therapy of cancers based on the effects of IL-2 on T cells. The first concern is that under certain circumstances IL-2 can sensitise T cells for Fas-mediated activation-induced cell death (AICD). The second is that, as mentioned in the introduction, IL-2 is a crucial factor for the development, survival and function of Tregs (i.e., cells that suppress the function of other T cells).

AICD predominantly affects $CD4^+$ T cells and occurs after T cell receptor stimulation [41]. It has been proposed that

AICD is most important for $CD4^+$ T cells that are repeatedly stimulated by persisting antigens, such as self or tumour antigens [42]. In contrast to the negative effect of IL-2 on $CD4^+$ T cells via AICD, IL-2 generally has a stimulatory influence on $CD8^+$ T cells. Thus, IL-2 production by $CD4^+$ T cells can be critical for the maintenance of tumour/self-reactive $CD8^+$ T cells [17,43]. These opposing effects of IL-2 on different T cell subsets are clearly a complication for immunotherapy.

$CD4^+$ T cells activated in the absence of IL-2 signals seem to be less sensitive to Fas-mediated AICD [44]. This might be due to inefficient degradation of inhibitors of apoptosis (such as c-FLIP) downstream of Fas [45]. Unlike IL-$2^{-/-}$ mice, IL-2 transgenic mice show no gross abnormality and contain increased numbers of Tregs [17,21]. It would be of interest to test whether IL-2 transgenic mice exhibit increased AICD.

3.3 $CD4^+$ $CD25^+$ T regulatory cells

The contribution of $CD4^+$ T cells to anticancer immunotherapy is controversial. On the one hand, $CD4^+$ T cell help can be beneficial for the priming and maintenance of $CD8^+$ T cell responses. Conversely, $CD4^+$ T cells include $CD25^+$ Tregs, which are immunosuppressive. Interestingly, there are cases of patients who initially respond well to IL-2 treatment, but later become refractory to subsequent treatment with IL-2, which might be due to the stimulation and expansion of Tregs by such treatment [21]. In fact, it has been shown that IL-2 administration does lead to expansion of Tregs in humans [46,47]. Furthermore, even tumour-infiltrating lymphocytes, which are thought to contain the most efficient tumour-reactive effector $CD8^+$ T cells, have been shown to contain functional $CD4^+$ $CD25^+$ Tregs [48]. Thus, there is considerable uncertainty as to whether IL-2-based treatment is of real benefit to anticancer immunotherapy. The use of other stimulatory cytokines, such as IL-7, -15 or -21, has been suggested because these cytokines act on cytotoxic T cells, but not Tregs [21,49]. Alternatively, depletion of Tregs (and other possibly suppressive and cytokine-consuming cells) by non-myeloablative chemotherapy prior to transfer of *in vitro* activated autologous tumour-reactive T cells has been explored successfully in patients with metastatic melanoma [50].

4. Modified strategies of IL-2 administration

Faced with the problem of IL-2 therapy-induced toxicity, researchers have begun to explore methods for avoiding this problem. One approach is to prolong the *in vivo* half-life of IL-2 using carrier proteins. Another method is to generate mutated versions of IL-2 that exhibit less VLS, or to use biological agents to reduce VLS. These topics are discussed below.

4.1 IL-2 carriers

Unbound/free IL-2 has a very short half-life in the serum of mice, ranging from 3 – 5 min following an intravenous injection, although intraperitoneal or subcutaneous administration can slightly prolong its half-life [51]. IL-2 is cleared mainly in the kidneys by degradation rather than simple excretion, whereas uptake by lymphoid cells does not seem to contribute to IL-2 clearance [51,52], which is somewhat surprising.

The first efforts to prolong the half-life of IL-2 *in vivo* involved coupling to a gelatine base, which provided promising results [51]. Subsequently, the use of IL-2–liposomes or a serum albumin–IL-2 fusion protein led to an increase in half-life and improved distribution to the liver, spleen and lymph nodes [53-55]. However, conclusive data on *in vivo* efficacy or toxicity of these conjugates are missing at present.

Another approach to increasing the half-life of IL-2 was to couple it to an unrelated antibody or antibody fragment. One group used an IgG3–IL-2 fusion protein and observed an *in vivo* half-life of this fusion protein that was ~ 10 times higher than free IL-2 [56]. A similar beneficial effect of conjugating IL-2 to an irrelevant IgG2b molecule was reported by another group [57]. Using their IL-2–IgG2b fusion protein, the researchers did not observe any obvious toxicity, although a detailed analysis of signs for toxicity was not performed. Moreover, numbers of $CD25^+$ $CD4^+$ Tregs increased considerably after administration of IL-2–IgG2b fusion protein. A third approach was to engineer a fusion protein of IL-2 with an antibody specific for a tumour antigen. Therefore, the half-life of IL-2 was prolonged, and IL-2 was directed to the tumour site [58,59]. As discussed in more detail later, another method using antibodies consists of combining an anti-IL-2 mAb with recombinant IL-2. As with the above, this approach prolongs the half-life of IL-2 *in vivo* [22]; however, the stimulatory effects on T cells are quite different.

4.2 Modified IL-2 proteins and IL-2 modifiers

The second line of research was designed to identify which part of the IL-2 molecule is responsible for VLS. These approaches rested on the assumption that the IL-2 epitope responsible for its therapeutic effect(s) is different from that causing VLS. A mutant IL-2 protein (termed IL-2 mutein) was generated by modifying the IL-2Rβ binding region of IL-2 at position Asn88 [60]. IL-2 mutein exhibited 3000-fold selectivity for overtly activated T cells over resting NK cells *in vitro* because IL-2 mutein bound activated T cells expressing high-affinity IL-2R, but not NK cells expressing intermediate-affinity IL-2R. IL-2 mutein was relatively non-toxic yet maintained considerable antitumour activity *in vivo*. Another group compared a mutation of that same amino acid residue at position Asn88 with a point mutation of Arg38 of IL-2, and came to the conclusion that the latter IL-2 mutant protein showed less VLS yet retained stimulatory immune function [59]. Likewise, a serine substitution at amino acid position 125 of IL-2 was reported to ameliorate IL-2 toxicity while not affecting its therapeutic effects [61]. Other

researchers were able to link the IL-2-associated VLS to a three-amino acid sequence at positions 19 – 21 of IL-2, and deletion of this sequence led to a marked reduction of damage to vascular endothelial cells [40]; however, this deleted sequence involved residue Asp20, which is crucial for the interaction of IL-2 with IL-2Rβ.

Another approach consisted of the generation of a synthetic non-peptidyl mimic of superoxide dismutase, which reduced IL-2-induced superoxide-mediated hypotension [62]. Interestingly, this treatment also increased IL-2-mediated antitumour effects through the inhibition of macrophage-derived toxic superoxide production, which negatively influences NK cell function. Interestingly, the addition of histamine improved IL-2 immunotherapy in mice [63], and histamine is known to inhibit the release of reactive oxygen species [62]. Recently, murine IL-2 was combined with murine IL-18 to generate a fusion protein with enhanced *in vivo* antitumour activities, but reduced toxicity in comparison with IL-2 alone [64].

5. Anti-IL-2 monoclonal antibodies

In the early 1990s, it was realised that complexing cytokines with anticytokine mAbs could prolong the half-life of the cytokine in serum [65]. Such bound cytokines could maintain their *in vivo* activity for a couple of days compared with just a few minutes for free cytokine alone. Prolonging cytokine half-life with mAbs applied to several cytokines, including IL-2 [66,67]. In this respect, the administration of IL-2/anti-IL-2 mAb complexes proved beneficial for tumour immunotherapy, although this effect was limited to a mild increase in NK cell-mediated tumour rejection.

Recently, it has been found that the combination of recombinant IL-2 with certain anti-IL-2 mAb leads to massive proliferation of antigen-primed (memory) $CD8^+$ T cells and also NK cells [22]. In contrast to these 'stimulatory' mAbs, other anti-IL-2 mAbs combined with recombinant IL-2 did not stimulate memory $CD8^+$ T cells and NK cells, but instead led to a three- to fourfold expansion of $CD4^+$ $CD25^+$ Tregs. The authors refer to these latter anti-IL-2 mAbs as 'inhibitory' mAbs.

5.1 Stimulatory antibodies

When combined with recombinant mouse (rm)IL-2, antimouse IL-2 mAb S4B6 and related mAbs preferentially stimulate the proliferation of memory $CD8^+$ T cells as well as NK cells *in vivo* [22]. Thus, after a course of daily injections of rmIL-2 plus S4B6 anti-IL-2 mAb for 1 week, an expansion in memory $CD8^+$ T cells of up to 100-fold was observed, and NK cell numbers increased by 20- to 30-fold. Conversely, $CD4^+$ $CD25^+$ Tregs responded more weakly to such treatment, resulting in three- to fourfold higher numbers compared with baseline. These results on the stimulatory ability of the S4B6 anti-IL-2 mAb combined with rmIL-2 for the proliferation of memory $CD8^+$ T cells and NK cells were subsequently confirmed by two other groups [20,68]. Stimulatory function also applied to an anti-IL-2 mAb specific for human IL-2, which was able to expand memory $CD8^+$ T cells and NK cells when combined with rhIL-2 [22].

Increases in the biological activity of cytokines after association with mAbs have been reported for several other cytokines, including IL-3, -4, -6 and -7 [22,65,69,70]. Several mechanisms have been suggested to account for the stimulatory effect of cytokine/anticytokine mAb complexes, including: prolonging the half-life of the cytokine by acting as a carrier protein and/or by reducing its renal filtration; more efficient cytokine presentation by binding to Fc receptor-bearing cells [$F(ab')_2$ mAb fragments were much less stimulatory than whole mAbs]; and inhibition of modification of the cytokine's active site [22,69]. With regard to the latter possibility, one study found that stimulatory function applied to a neutralising anticytokine mAb, but not to a non-neutralising mAb [69], suggesting that serum or blood may contain proteolytic enzymes that rapidly inactivate the cytokine's receptor binding site after its secretion. For mouse IL-2, however, such inhibitory activity could not be found in mouse serum or blood [51].

5.2 Inhibitory antibodies

Compared with the above-mentioned stimulatory anti-IL-2 mAbs, very different effects were observed with another anti-IL-2 mAb, JES6-1. When this inhibitory anti-IL-2 mAb was complexed with rmIL-2, the complexes were unable to expand memory $CD8^+$ T cells or NK cells, but retained their ability to stimulate $CD4^+$ $CD25^+$ Tregs [22].

The opposing functions of stimulatory and inhibitory anti-IL-2 mAbs correlate with differential IL-2R expression pattern on the affected lymphoid subsets. Whereas memory $CD8^+$ T cells and NK cells express very high levels of CD122 and only background levels of CD25, Tregs show very high expression of CD25 and intermediate levels of CD122; expression levels of γ_c are comparable on these cell types. The point to emphasise is that memory $CD8^+$ T cells and NK cells express high levels of the intermediate-affinity IL-2R, whereas Tregs carry all three subunits necessary for the high-affinity IL-2R. Due to their lower-affinity IL-2R, memory $CD8^+$ T cells and NK cells are less sensitive to unbound/free IL-2 than Tregs. However, when IL-2 immune complexes containing stimulatory mAbs are used, memory $CD8^+$ cells and NK cells are favoured over Tregs. Why? One reason might be that stimulatory anti-IL-2 mAbs bind to a region of the IL-2 molecule, which is important for CD25 binding, but not for CD122 (**Figure 2**). Conversely, inhibitory mAbs may cover an IL-2 region crucial for CD122 interaction without affecting CD25 binding; thus, the only cells responsive to immune complexes of rmIL-2 with inhibitory anti-IL-2 mAb are $CD25^+$ Tregs, $CD122^+$ $CD25^-$ memory $CD8^+$ cells and NK cells being unable to recognise these complexes [22].

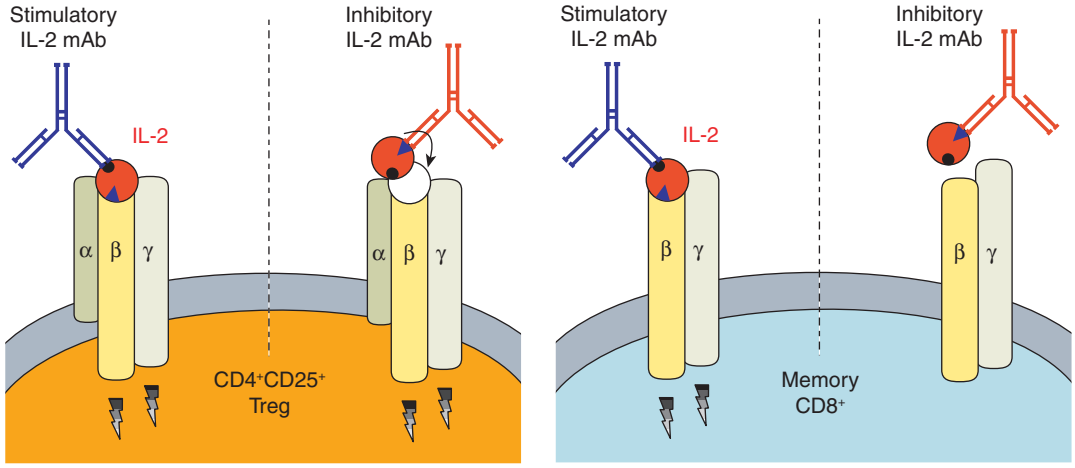

Figure 2. Selective stimulation of T cell subsets with different IL-2/anti-IL-2 mAb immune complexes. Stimulatory anti-IL-2 mAbs are able to stimulate cells expressing high-affinity IL-2R (such as CD4+ CD25+ Tregs) and cells expressing only intermediate-affinity IL-2R (such as memory CD8+ T cells) because they presumably bind to a region of the IL-2 molecule which is important for CD25 binding (dot in IL-2 molecule), but not for CD122 (triangle in IL-2 molecule). Conversely, inhibitory anti-IL-2 mAbs may cover an IL-2 region crucial for CD122 interaction without affecting CD25 binding; thus, the only cells responsive to immune complexes of IL-2 with inhibitory anti-IL-2 mAb are CD4+ CD25+ Tregs. Memory CD8+ cells, which do not express significant levels of CD25, are unable to recognise these complexes.
Treg: T regulatory cell.

6. Use of anti-IL-2 antibodies in immunotherapy

As discussed above, based on the choice of anti-IL-2 mAb, one can either preferentially stimulate memory CD8+ T cells and NK cells, or CD4+ CD25+ Tregs.

6.1 CD8+ T cell- and NK cell-directed immunotherapy

Tipping the balance between CD8+ T cells and CD4+ CD25+ Tregs towards the former might be quite useful for immunotherapy directed against persisting foreign or self-antigens, which is the case with chronic viral infections and tumours. In accordance with this idea, a course of rmIL-2 plus S4B6 anti-IL-2 mAb was more protective in a model of lung tumour metastasis than either treatment alone [68].

It is notable that administration of a stimulatory anti-IL-2 mAb can expand numbers of memory CD8+ T cells even when injected alone. In this situation, the injected mAb forms complexes with endogenous IL-2, these complexes being strongly stimulatory for memory CD8+ T cells [22,71]. In low concentrations, however, these complexes are poorly stimulatory for cells expressing high-affinity IL-2R, such as Tregs. For these cells, anti-IL-2 mAb injected alone serves to neutralise IL-2 and thereby depletes Tregs. Hence, by stimulating CD8+ T cells and depleting Tregs, injecting anti-IL-2 mAb alone could be an effective approach for immunotherapy. A further possibility consists of the combined administration of stimulatory anti-IL-2 mAb, in order to reduce Treg numbers, together with IL-15, which stimulates memory CD8+ and NK cells, but not Tregs. This combination was used successfully in a murine model of tumour immunotherapy [17], and it would be interesting to directly compare this approach with the use of stimulatory IL-2/anti-IL-2 mAb immune complexes.

Further use of stimulatory IL-2 immune complexes could be envisaged during the effector and contraction phase of the T cell response. Thus, injecting unbound/free IL-2 was shown to considerably decrease T cell death at the end of an antiviral response, thereby leading to higher numbers of long-lived memory CD8+ T cells [18]. In addition, exposure to IL-2 during the initial expansion phase of an antiviral CD8+ T cell response was shown to be crucial for endowing memory CD8+ T cells with efficient proliferative capacity following second antigen challenge [20]. Thus, administration of stimulatory IL-2 immune complexes during the initial phase of a CD8+ T cell response could be useful for generating higher numbers of long-lived memory T cells.

It should be mentioned that a course of IL-2 plus stimulatory anti-IL-2 mAb immune complexes for 1 week does not cause any obvious signs of toxicity in mice; however, unlike in humans, one has to examine carefully for signs of toxicity in mice, such as pulmonary oedema, pleural effusions and ascites, among others. Thus, it will be crucial to test specifically for toxicity induced by IL-2/anti-IL-2 mAb immune complexes and compare to free IL-2.

6.2 CD4+ CD25+ T regulatory cell-directed immunotherapy

Several studies have shown a correlation between the incidence of autoimmune disease and a reduction of Tregs. The first evidence here came from analyses of IL-2$^{-/-}$, CD25$^{-/-}$ and CD122$^{-/-}$ mice, which all lack typical CD4+ CD25+ Tregs and develop symptoms of autoimmune disease. More recently, other reports showed that autoimmune disease develops after mAb-mediated ablation of Tregs [4]. Thus, increasing numbers of CD4+ CD25+ Tregs (e.g., by administration of IL-2) might be useful for the treatment of autoimmune diseases. The problem here is that IL-2 also stimulates CD8+ T cells, which could intensify autoimmunity; however, this problem could be avoided by injecting IL-2 complexed with the inhibitory anti-IL-2 mAbs considered earlier, thus exclusively stimulating Tregs without influencing proliferation of CD8+ T cells or NK cells. Whether this approach is indeed useful for the treatment of T cell-mediated autoimmune diseases has yet to be tested.

7. Expert opinion and concluding comments

IL-2-based treatment strategies for cancers and immunodeficiency have been used for more than 2 decades, but with severe side effects. More recently, IL-2-mediated stimulation of Tregs has become a major concern in IL-2-based therapies. This latter issue might now be addressed by the use of stimulatory IL-2 immune complexes. This approach will have to be compared with other methods for reducing or avoiding the stimulation of Tregs, as discussed above. A crucial issue is whether IL-2–anti-IL-2 mAb immune complexes are less toxic than IL-2 given alone. If VLS increases in parallel with enhanced immune stimulation, then IL-2–anti-IL-2 mAb immune complexes might be just as toxic as free IL-2. There are, however, several reports suggesting that the therapeutic effect of IL-2 is mediated by a different site on the IL-2 molecule than the epitope responsible for VLS. Direct evidence on whether IL-2/anti-IL-2 mAb complexes are less toxic than free IL-2 will have to await further investigations.

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