Anti-cytokine Ab immune therapy: present status and perspectives

Daniel Zagury and Robert C. Gallo

Allergy, autoimmunity and the pathogenesis of some chronic diseases are dependent on host innate and adaptative immune responses. Both responses are associated with abnormal cytokine production within pathologic tissues. Over the past two decades, the availability of purified cytokines and cytokine antibodies (Abs) has prompted a therapeutic approach that aims to supply neutralizing Abs against deleterious cytokines, through either passive immunization (administration of large quantities of high affinity Abs, prepared *ex vivo*) or active immunization (induction of specific Abs, using immunogenic cytokine derivatives). Both passive and active immunization can safely, transiently and effectively be used, as has been documented by animal experimentation and confirmed by clinical trials. Novel anti-cytokine therapeutic compounds, based on passive Ab immunization, are now available to treat rheumatoid arthritis (RA) and have been shown to help control neoangiogenesis in cancer patients. Clinical trials using Abs to treat allergic disorders are also underway. However, the induction of anti-idiotypic Abs may restrict the long-term use of anti-cytokine immunotherapy using allogenic or humanized/chimeric Abs. We propose that greater consideration should be given to active immunization protocols.

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▼ The immune system not only exercises 'police activities' via a steady-state protection towards self antigens, it also assumes sentinel functions, leading to the rejection of pathogens, such as bacteria, viruses, fungi, parasites and pollen [1,2]. Both functions are performed by immune cells that comprise different types of T cells and antigen-presenting cells (APCs). These cells act in a well-coordinated but complex orchestration, mediated by cytokines.

Cytokines represent major signals that control tissue homeostasis by directing intracellular pathways in paracrine/autocrine target cells that carry specific receptors. In lymphoid tissues, cytokines regulate immune cell functions: activation, proliferation, differentiation, resting stages and cell death [3]. Their inducible secretion by effector cells is triggered by various stimuli, including microbial products, nucleotides (oligonucleotides; double-stranded RNA) and other cytokines. Recognition of released cytokines by specific membrane receptors induces a target cell response, at the completion of which a negative feedback regulation blocking the cytokine secretion by effector cells terminates the cytokine reaction [4]. Natural cytokine antagonists, including inactive cytokine receptor antagonists, such as IL-1ra, and soluble receptors, such as TNFα (tumour necrosis factor) and IL-4 (interleukin 4) receptors [3], do not appear to have a major role in the regulation of cytokine processes during pathophysiologic events. Also, antagonistic Abs to self cytokines, such as IL-1α, IFNα, IL-6, IL-10 and IFNγ (interferon-gamma) , can be detected in the sera of healthy individuals, but their clinical and biological relevance is still obscure [5]. These data are not surprising, given that anti-cytokine Abs secreting B cells, by contrast to T cells, have not been negatively selected and are still present, albeit relatively silent [6]. Furthermore, the capacity to raise levels of high affinity anti-cytokine Abs under pathophysiologic situations or following cytokine therapy is restricted, due to the absence of corresponding T helper (Th) cells [7,8]. In AIDS patients, the slight increase of specific anti-IFNα Abs, resulting from B cell polyclonal activation, is ineffective because these Abs are of low titer and are not neutralizing [9].

In various pathologies, including allergy, autoimmunity, cancer, AIDS and other infectious diseases, an abnormal release of cytokines contributes to pathogenesis and the spreading of disease [4]. Soluble receptors [10], Abs to cytokine receptors [11] and anti-cytokine Abs [12,13] have been administered to counteract cytokine-induced pathogenic

 1359-6446/04/$ – see front matter PII: S1359-6446(03)02955-6

effects. This study provides an overview of anticytokine Ab therapeutic approaches, based on experimental and clinical examples, rather than an exhaustive review of products on the market. We focus on how to achieve Ab immunization against deleterious self-cytokines and how and when it can be used safely and efficiently as a therapy.

This novel specific immune therapeutic strategy is founded on the concept that high affinity Abs, circulating through the stroma of pathologic tissues, antagonize the excess cytokine that impairs the natural and/or vaccine-induced immune reaction that is directed toward pathogenic entities, such as cancer cells, HIV-1 or HCV (Hepatitis C)-infected cells, or allergens. This concept has been validated by experimentation and clinical trials, which are discussed later. However, it raises an apparent paradox, in that it proposes vaccination against 'self' molecules. How can we selectively vaccinate against the undesirable consequences of cytokine activity in pathologic tissues, whilst maintaining physiologic functions in normal tissues? This question has been answered previously [13]. In brief, cytokines mediate their normal biological effects locally, between interacting effector-target cell types that make up a 'cytokine field' [14]. This is well illustrated by the 'immunological synapse' that is formed by a T cell and an APC, analogous to the classical synapse at the neuromuscular junction. The key point is that induced and homeostatically-regulated cytokines are locally produced, for local effects. The central hypothesis implies that high titer and high affinity Abs to a deleterious cytokine, when sufficiently concentrated in the abundant reservoir that is represented by stromal lymph flow of pathologic tissues, can locally neutralize the undesirable cytokine and reduce its pathogenic effects. By contrast, under physiological conditions, normal tissues exhibit negligible lymph turnover. Thus, the poorly renewed lymph Abs do not impair the homeostatically regulated cytokine reactions that occur within the immunological synapse, formed by the tight association of effector and target cells [13,14].

Generation of anticytokine antibodies for specific immunotherapy

High affinity, neutralizing Abs to cytokines can either be supplied by passive administration or induced by active immunization.

Passive anticytokine antibody immunization

For this purpose, monoclonal Abs, prepared by hybridoma technologies [15] and selected on the basis of their high neutralizing activity, are commonly used. Also, chimeric mAbs, constructed from variable regions of murine origin and constant regions from a human source [16], or humanized mAbs, constructed with antigen-binding regions (i.e. complementary determining regions (CDR), derived from a mouse, and constant regions, derived from a human source [16]) are currently available. Since 1997, three chimeric and five humanized mAbs have been licensed for human therapeutic use in America [16,17], and a more recently developed PEGylated (Polyethylene glycol) anti-TNFα antibody fragment (CDP-870), is currently in clinical trials [18]. A third approach involves the use of human anti-cytokine mAbs, obtained from the culture supernatant of clones of human B cells, immortalized by the Epstein Barr virus (EBV) [16]. Considering, however, that the yield of mAb production by B–EBV cell lines is not high, the therapeutic application of this technology appears to be limited. Finally, human mAbs have been investigated recently, using a single-chain Ab variable-region fragment (scFv) phage display library in which B cell clones producing mAbs are selected by biopanning [19,20]. A high-affinity human anti-TNFα mAb, derived from the blood cells of non-immunized individuals, has been produced by this technology [21], and the available supply of B cells from immunized individuals (see next section) should, in the near future, markedly improve the yield of human anticytokine mAb production.

Given their short half-life of three weeks, a high quantity of mAbs (5–15 mg kg^{-1}) is currently administered around two or three times per month, for the treatment of chronic diseases [22,23].

Active anticytokine antibody immunization

High neutralizing Ab titers can be triggered by active immunization, using kinoid immunogens [24], which are capable of breaking immunological B cell but not T cell tolerance to self cytokines. Such immunogens might be cytokine derivatives that are chemically coupled to a foreign Th carrier protein, such as tetanus toxoid or keyhole limpet haemocyanin (KLH). (These two carriers are FDA-approved proteins for clinical use). For cytokines that induce undesirable immune effects that can hamper or deviate immune reactions, such as immunosuppressive IFNα, TGFβ (transforming growth factor) or IL-10, the cytokine has to be chemically converted into a biochemically inactive, but still immunogenic, derivative before it is coupled to the carrier [24]. Genetically-modified kinoids, devoid of undesirable effects, can also be used. An anti-cytokine immunizing procedure that avoids cytokine coupling to a carrier protein might be applied to subjects exhibiting an abnormal B cell polyclonal activation, such as HIV-1-infected patients. For these individuals, the immunogen can be substituted by a non-toxic cytokine derivative immunogen, such as an IFNα kinoid, as currently used in clinical trials in AIDS patients, to combat HIV-1-induced immunosuppression [25].

Table 1. Characteristics of immunity that are conferred by anti-cytokine passive and active antibody (Ab) therapy, compared to conventional vaccination

	Passive Ab therapy	Active Ab therapy	Conventional vaccine
Current active principle	Humanized/chimeric Abs	Cytokine derivatives	Foreign antigen
Medical use	Therapeutic	Therapeutic	Preventive/therapeutic
Abs to pathogenic Ag	Monoclonal	Polyclonal	Polyclonal
Serum neutralizing Ab titers at peak	High	High	High
Specific cellular response	None	None	Yes
Effective immunity	Immediate	3–4 weeks	3–4 weeks
Decline of Ab immunity	3 weeks	3–6 months	Years
Booster injections	1–2 per month	2–4 per year	1–10 years
Anti-Ab response	Following repeated injections[a]	None	None
Risk of relapse	Following repeated injections[a]	None	None
Patients compliance	Cumbersome	Well tolerated	Well tolerated
Cost	High	Low	Negligible

[a]Specific anti-idiotypic Abs to passively administered anti-cytokine Abs can be induced by repeated booster injections

For active anti-cytokine Ab treatment of chronic diseases, 2–4 priming injections of kinoid in incomplete Freund's adjuvant (IFA; FDA-approved, ISA51), at weekly or monthly intervals, elicited a robust humoral response, with high-affinity neutralizing Abs but no cellular response to the cytokine, both in mice and humans [7,8,13,25]. The anticytokine antibody response was transient, lasting 3–6 months, as anticipated, given that Ab production by activated B cells in the absence of specific T cell help is limited to ~12 weeks [26,27]. However, to maintain long-term effective neutralizing Ab levels in the body fluid, booster injections with the kinoid can be repeated when Ab titers decline [25]. Of note in vaccine procedures comprising a foreign carrier protein, a collateral cellular response to the carrier is usually observed without any long-term medical consequences.

Table 1 summarizes the characteristics of passive and active immunization procedures relevant to their therapeutic use. In addition, Figure 1 shows that, in mice immunized with muIL4 kinoid, two weeks after a booster injection, the capacity of 1 ml serum to neutralize muIL4 activity, as assessed by the standard functional test using HT-2 cells [28], is equivalent to that of ~2-3 mg mAb, selected for their ability to neutralize muIL-4 (R&D, MAB404).

Side effects associated with antibody therapy

The safety of anticytokine Ab therapy is supported by documented experimentation and clinical trials [13]. Administration of mAbs to cytokines such as anti-IL4 [29,30], anti-IL5 [31], anti-IL6 [32,33], anti-TNFα [22,34], anti-VEGF (vascular endothelial growth factor) [23] mAbs to rodents and/or humans is innocuous. Active immunization, eliciting high neutralizing Ab titers against various cytokines, including EGF (epidermal growth factor) in adult rats and humans [35,36], IL1 [37], IL9 [7] and TNFα in mice [8] and IFNα in humans [25,38], is safe. However, anti-EGF immunizations, performed in pregnant female rats triggered teratogenic lesions in the embryo [35].

Although experimental and clinical trials confirmed that both passive and active immunizing procedures neither impaired the physiology of normal tissues [4] nor triggered adverse autoimmune disorders [13], they, nonetheless, exposed some patients to specific side effects (Table 2). In the most advanced trial, namely one directed against TNFα, adverse side effects were chiefly infections or the worsening of heart failure in cardiac patients (Table 2) [22,39]. In a successful Phase III trial, in which anti-VEGF mAbs (Bevacizumab) were repeatedly administered to colorectal cancer patients, the major reported adverse side effect was grade III hypertension in 11% of patients (Table 2). However, this complication was well managed by oral medication [40].

A limitation of passive immunization that was discovered following the current use of heterologous chimeric or humanized mAbs was the production of anti-anticytokine Abs (anti-idiotype Abs). This has been reported in the treatment of Castelman's disease with murine anti-IL6 Abs [32] and has also been observed following repeated injections of TNFα mAbs in 40% of rheumatoid arthritis (RA) patients and 13% of Crohn's disease patients [22].

Indications of anti-cytokine Ab immune therapy

The efficacy of specific anticytokine Ab immunizations has been documented by animal experiments [7,8,29,30,34]

and, in some instances, clinical trials [22,38,40]. Specific Ab immunization might, indeed, represent a potent specific therapy for combating dysregulated cytokines that are associated with damaged tissues in chronic diseases if the relevant cytokine target, pivotal for the local cytokine network impairment, can be identified.

Cancer

In malignant tumors, neoangiogenesis and immunosuppression are common stromal characteristics, mediated by locally-released cytokines (Figure 2a). These pathogenic features result from the vicarious release by cancer cells of regulatory factors, including cytokines themselves [41,42], or viral factors, such as the E7 protein, derived from HPV-16 in uterine cervical carcinoma [43,44].

Neoangiogenesis

Angiogenic VEGF is released by culture supernatants of freshly isolated cells from primary tumors and cancer cell lines (Figure 2a) [45], the effects of anti-VEGF Ab administration was tested in cancer patients, and the Phase III trial, conducted on colorectal cancer patients, was successful after 18 months [40].

Considering the risk of raising an anti-idiotypic Ab response to chimeric Abs, we have proposed an active vaccine approach as an alternative to passive immunization. The kinoid consists of a KLH–VEGF immunogen that induces high titers of neutralizing Abs. For the preclinical experimental phase of this project, a KLH–murine VEGF kinoid in ISA 51 was administered to mice and the vaccine preparation was shown to be innocuous over the lifetime of the mice (18 months). Also, it triggered high titers of anti-VEGF neutralizing Abs (Figure 3). A similar KLH–huVEGF kinoid is now ready to enter Phase I clinical trials.

Immunosuppression

TGFβ and/or IL-10 are abnormally released by freshly isolated cells and cell lines from various tumors, including uterine cervical carcinoma and ovarian cancer (Figure 2a) [13]. The release of immunosuppressive cytokines in the stromal microenvironment is likely to favor local immune suppression. Anti-TGFβ or IL-10 Ab immunization, either passive or active, should represent an effective approach against this immune escape strategy of the cancer cell, and we propose that this approach should also be clinically tested in conjunction with conventional therapy, as for anti-VEGF Ab immune therapy. As a point of interest, both anti-TGFβ–1 (metelimumab) [46] and –2 (lerdelimumab) [47] (Cambridge Antibody Tech.; http://www.cambridgeantobody.com), and a murine anti-IL-10 (B-N10; Diaclone; http://www.diaclone.com) [48] mAbs immune therapy have already entered clinical development stages, but for indications other than cancer – for the prevention of post surgical fibrosis in glaucoma and systemic lupus erythematosus (Table 2).

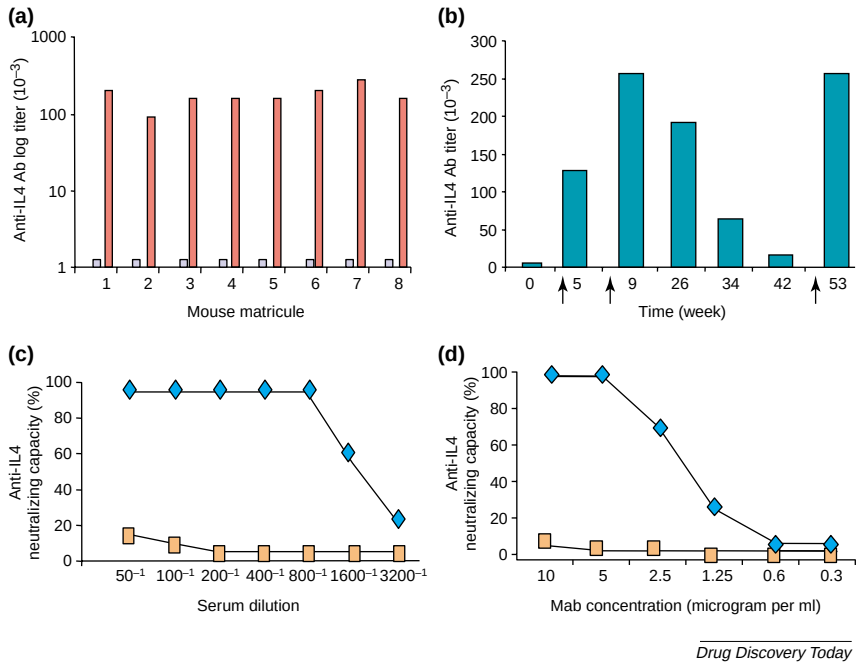

Figure 1. Humoral response of mice immunized with murine IL-4 kinoid. **(a)** Peak of response in a series of immunized mice expressed as the anti-IL-4 Ab levels assessed by ELISA, using muIL4 (50 ng/well) coating and mice sera ($1/10^6$ to $1/10^9$ dilutions). Cut-off of positivity corresponded to 0.3 Optical Density (O.D.), negative control serum being ≤0.01 O.D. Pre-(▫) and post- (▪) immunization sera. **(b)** Kinetics of the serum Ab response of a representative immunized mouse by ELISA. Samples were collected at various times of the immunizing procedure. Booster injections are indicted by arrows. **(c)** Anti-IL-4 neutralizing capacity of serum from immunized mice (◆), in comparison with control serum (▫) and **(d)** Anti-IL-4 neutralizing capacity of anti-IL-4 mAb (◆) (MAB 404, R&D) compared to control murine IgGs. (▫) mu IL4 biological assay was performed using HT2 cells (American Type Culture Collection, a murine T helper cell line, responsive to murine IL4) as described by Watson *et al.* [26]. Cells were pretreated with murine IL-4 (60 ng ml^{-1}) and cultured 1c) in the presence of various dilutions of serum from immunized and control mice or 1d) in the presence of serially diluted monoclonal antibody (MAB-404, R&D), selected for their neutralizing capacity and control murine IgGs. Cultured cells were pulsed with [3H]-thymidine and processed for measuring radioactivity in a β counter.

Table 2. Anti-cytokine antibody (Ab) immune therapy in clinical trials: active principle and major adverse side effects

Active principle	Generic name[b]	Major indication	Target	Development stage	Major side effects
Anti-TNFα mAbs	Infliximab	Rheumatoid arthritis and Crohn's disease	Inflammation	Approved for marketing	Infections including tuberculosis, worsening of heart failure [22,39]
	Adalimumab (D2E7)	As above	As above	Approved for marketing	As above [21]
	CDP571	As above	As above	Discontinued	As above [63]
	CDP-870	As above	As above	Phase III	As above [18]
Anti-VEGF mAbs	Bevacizumab	Cancer (colorectal renal and others)	Neoangiogenesis	In US regulatory review	Grade III hypertension [40]
Anti-IL-4 mAbs	Pascolizumab	Allergy (Asthma)	IgE switch	Discontinued	Not reported [30]
Anti-IL-5 mAbs	Mepolizumab	Allergy (Asthma)	Eosinophilia	Clinic	Not reported [31]
Anti-IL-6 mAbs	CNTO-328	Multiple myeloma	Ig overproduction	Clinic	Not reported [33]
	-	Castleman disease	Ig overproduction	Phase I	Disease relapse [32]
Anti-TGFβ1 mAbs	Metelimumab	Diffuse systemic sclerosis	Phase I/II	Not reported [46]	
Anti-TGFβ2 mAbs	Lerdelimumab	Prevention of post chirurgical fibrosis	Wound healing	Phase III	Not reported [47]
Anti-IL10 mABs	B-N10	Systemic Lupus Erythematosus	Immunosuppression	Clinic	Anti-idiotype Abs [48]
IFNα kinoid	Antiferon	AIDS	Immunosuppression	Phase II	Not reported [25]
TT / P64K-EGF[a]	-	Cancers	Epithelial cell growth	Phase I	Not reported [36]
EGF in CFA	-	Cancers	Epithelial cell growth	Preclinical	Teratogenic lesions in embryos of pregnant rats [35]

[a]TT (Tetanus toxoid) and P64K (Neisseria Meningitis) carrier proteins to EGF.
[b]Infliximab was manufactured by Centocor (Remicade®) [22], D2E7 by CAT and Abbott (Humira™) [21], CDP571 by Celltech [63], CDP-870 [18] by Celltech/Pfizer Bevacizumab by Genentech [40], Pascolizumab [30] and Mepolizumab [31] by Glaxo Smith Kline, CNTO-328 [33] by Centocor, Metelimumab [46] and Lerdelimumab [47] by CAT, B-N10 [48] by Diaclone and Antiferon™ [25] by Neovacs.

Microbial infections

Pathogens have evolved disparate advantageous strategies to escape the immune system, including the differentiation of suppressive T cells (Tr1), which limit the protective ability of immune reactions by allowing long-term infection of the host. Examples of these abound. Bacteria such as *Bordetella pertussis* present a filamentous hemagglutinin protein that inhibits IL-12 and enhances IL-10 production from dendritic cells (DCs) [2]. Whereas the majority of virus species, including vaccinia, are contained by the host immune system and effector cytolytic T lymphocytes (CTLs), in particular (Figure 2b), other viruses, such as HIV-1 [49], HPV-16 [43,44] and EBV [50], inhibit cellular immune responses. This immune suppression is mediated by cytokines, particularly IFNα and IL-10, which are either directly produced by infected cells (e.g. viral IL-10, produced by EBV-infected cells [50]) or triggered by extracellular viral factors that are released by infected cells (e.g. the Tat protein of HIV-1 [51] – Figure 2b – or the E7 protein of HPV-16 [43]).

IL-10 is the pivotal immunosuppressive cytokine and its production by Tr1 cells is triggered by IFNα [52]. IFNα, a major antiviral factor of innate immunity, produced by APCs and, primarily, plasmocytoid dendritic cells type 2 (DC2), also has a key role, although pleiomorphic, in adaptative immunity [13]. It acts, initially, as an inducer of T cell differentiation but at the terminal stage of the immune reaction, it is immunosuppressive, acting in synergy with IL-10 as a negative feedback regulator of the immune reaction [52]. The immunosuppressive effects of IFNα can be evaluated *in vitro*, following Staphylococcus aureus

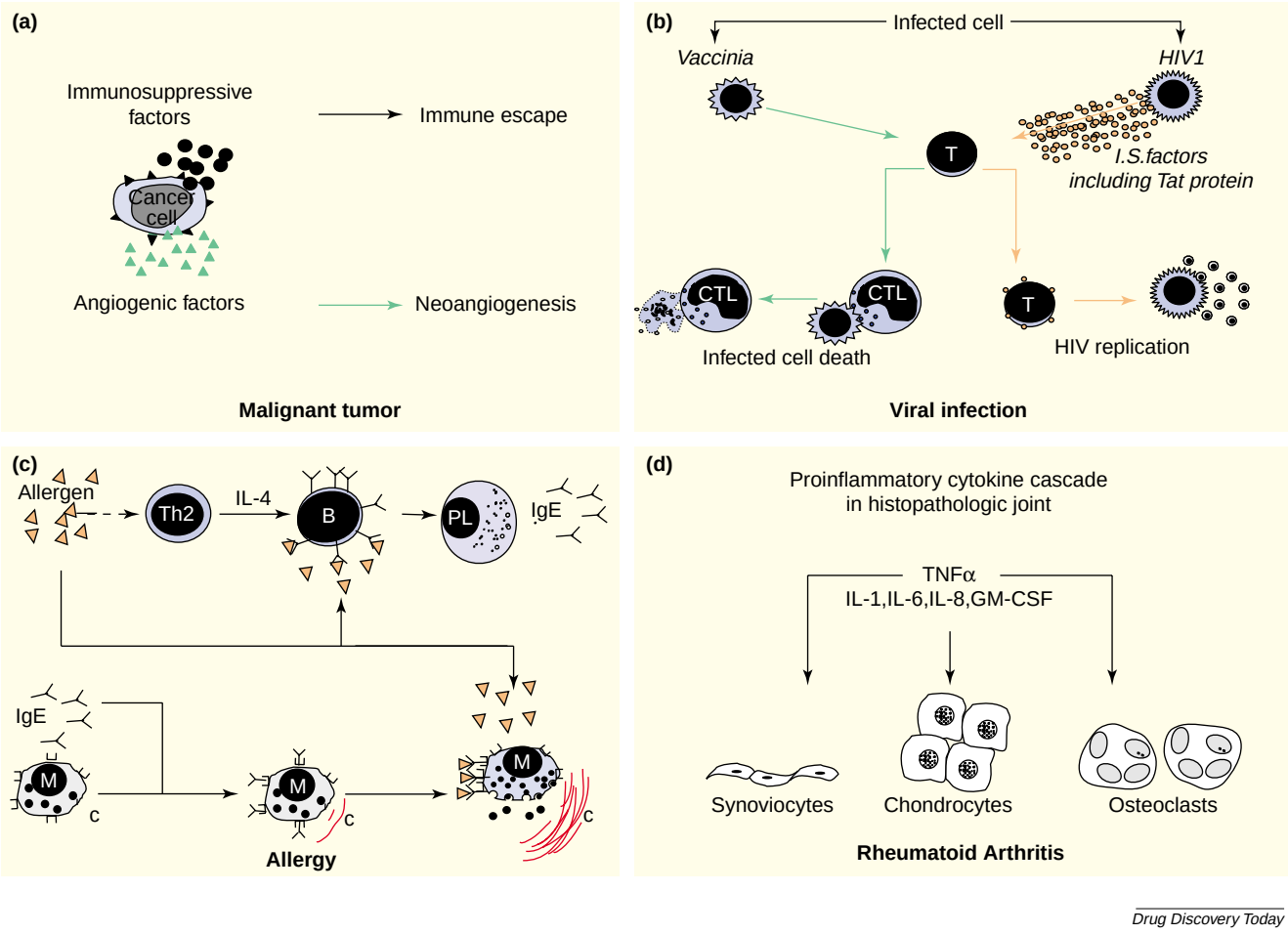

Figure 2. Representation of the mechanisms underlying the histopathology of cytokine-dependent chronic diseases. **(a)** In the microenvironment of malignant tumors, immunosuppression and neoangiogenesis are common stromal features, mediated by cytokines [13,41,42]. **(b)** In viral infections, while vaccinia virus triggers effective cytolytic T lymphocytes (CTL), immunosuppressive factors released by HIV-1-infected cells inhibit cellular immune reaction [49]. **(c)** In allergy, IgE-induced inflammatory reactions are triggered by allergen-induced IL4 overproduction [59]. **(d)** In RA, proinflammatory cytokines overproduction is orchestrated by TNFα [61]. c, capillaries; IS, immunosuppressive; M, mast cells; PL, plasma cells.

enterotoxin B or recall antigen (purified protein derivative or Tetanus toxoid) stimulation of cultured Peripheral Blood Mononuclear Cells (PBMCs). T cell activation is inhibited in a dose–effect manner, as measured by T cell proliferation and by IL-2 production [53,54]. Furthermore, the immune-suppressive role of IFNα might also be documented *in vivo* because, in the absence of IFNα during the resolution of influenza viral diseases [55], the host suffers from detrimental effects, triggered by an excessive cellular immune response. Targeting immune suppression by vaccination against these relevant pathogenic immunosuppressive cytokines will enhance the capacity of the host immune system to contain the infection.

During HIV infection, progressive cellular immunosuppression (Figure 2b) is associated with increasing titers of circulating IFNα. Indeed, high titers of serum IFNα represent a marker of progression towards AIDS [56]. The role of IFNα as a mediator of HIV-1-induced immunosuppression is further supported by experiments that demonstrated the prevention of suppressive Tr1 generation by anti- IFNα Abs added to HIV-1-infected PBMC culture cells [49]. Thus, anti-IFNα Ab immune therapy appears to be, perhaps, 'the' appropriate means of controlling HIV-1-induced immune suppression and the evolution to AIDS. Active anti-IFNα Ab therapy trials have already been conducted, initially by Gringeri *et al.* [25], followed by a Phase II EURIS trial (European-Israeli Trial) [38]. These trials suggested that the treatment was safe and provided some benefit to the immunized patients, therefore, a multicentric Phase III trial was deemed appropriate and is currently in preparation.

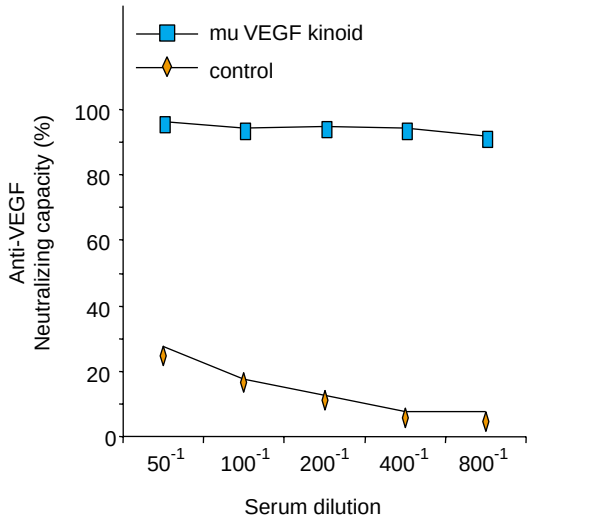

Figure 3. Anti-VEGF neutralizing capacity was measured by inhibition of human umbilical vein endothelial cells' (HUVEC) proliferation by anti-VEGF Abs. Subconfluent HUVEC (5×10^3 cells/well) were seeded into 96-well plates in endothelial cell growth medium (M199), containing 5% foetal calf serum. After 24 hours, the growth medium was replaced by the corresponding serum-free medium, supplemented with 1.5% foetal calf serum, and 30 ng ml^{-1} murine VEGF, pretreated with varying dilutions of serum from immunized or control mice. Endothelial cell proliferation was assessed by [3H]-thymidine incorporation and the results are expressed as % of inhibition of cell proliferation.

Two additional observations (*inter alia*) also add to this rationale. First, it has been shown that HIV-1-induced thymic deficiency is due to IFNα overproduction [57], and second, HAART-induced lipodystrophia is associated with increased levels of circulating IFNα [58].

Allergy

Abnormal production by Th2 cells of the B cell costimulus IL-4, following exposure to an allergen, induces high titers of circulating Abs of the IgE class, triggering allogenic inflammatory reactions (Figure 2c) [59]. Supply of high-affinity anti-IL-4 Abs, antagonizing the overproduced cytokine, should contain the Th2 immune deviation and subsequent allergic disorders. This assumption was based on animal experimentations, showing that passive administration of either anti-IL-4 mAbs [29] or IL-4 receptor antagonists [60] block allergic reactions. These data explain: 1) why a specific immune therapy, using a chimeric mAb (pascolizumab) has been tested in asthmatic patients up to Phase 2 (Table 2) [30] and; 2) why data on IL-4 immunized mice (Figure 1) prompted the planning of a clinical trial to test a hu IL-4 kinoid immunogen that has triggered high titers of Abs that neutralize hu IL-4 biological activity in mice. Interestingly, in preclinical studies in mice, we found that neutralizing Abs to murine IL-4 (Figure 1) reduced the allergen (Betv1)-induced IgE Abs levels and inhibited the passive cutaneous anaphylactic reaction (PCA) in birch pollen-sensitized animals (not shown).

Autoimmunity

Autoimmune RA disease is associated with an overproduction of proinflammatory cytokines in response to environmental factors (Figure 2d). These cytokines include TNFα, IL-1, IL-6, IL-8 and GM-CSF [61]. In cultured synovial cells from patients with RA, the presence of anti-TNFα Abs neutralized the corresponding cytokine and further markedly down-regulated IL-1 production and GM-CSF, IL-6 and IL-8 [62]. Thus, the impaired proinflammatory cytokine cascade that is seen in RA pathologic tissues appears to be triggered by an upstream overproduction of TNFα. These considerations prompted experiments that showed that both passive and active anti-TNFα immunizations were therapeutically effective in improving RA symptoms in animals [8,34]. Following these studies, clinical trials using massive dose of anti-TNFα chimeric Abs (infliximab) were undertaken [22]. Infliximab, given at 1 and 10 mg kg^{-1} in association with an immunosuppressive drug (methrotexate), was effective in 55% and 75% of RA patients. Furthermore, 82% of Crohn's disease patients receiving infliximab (5 mg kg^{-1}) responded to the treatment in comparison to 17% of the placebo group [22]. Notably, 19% of patients exhibited acute infusion reactions; however, given their clinical benefit, anti-TNFα mAbs are now commercially available as treatments for both Crohn's disease, particularly during acute ulcerative crisis, and RA [22,38]. The success of these trials led to multiple approaches by many groups, targeting TNFα, including mAbs, such as CDP 571 (Humicade™), which has now been discontinued, due to the severity of side effects (septic shock) [63], and D2E7 (Humira™) [21] (Table 2) or TNFα receptors fusion proteins, such as Lenercept [64] (Roche; discontinued) and Etanercept (Enbrel®) [65]. In this context, we (D.Z.) have produced a TNFα kinoid immunogen, which is now in pre-clinical development.

General contraindications to antibody immune therapy

The therapeutic use of anti-cytokine Ab immunization (passive or active) to combat a disease that is associated with a particular deleterious cytokine requires successful preclinical data, followed by well-monitored clinical trials. Given the results described here, this approach, particularly when dealing with growth factors (EGF, VEGF) [35,66], should not be used to treat pregnant women because

of the risk of teratogenicity on progeny. Antibody immune therapy is also contraindicated in patients presenting a comorbid condition in which reduction of the given cytokine might be clinically inappropriate [13]. Finally, individuals presenting a positive skin prick test to the immunogen should also be excluded from active immunization, to avoid the risk of an allergic reaction, as should those for whom active vaccine is contraindicated (e.g. patients suffering from cachexia, nephropathy or patients receiving immunosuppressive medication, including corticoids and β-blocking drugs).

Discussion

The availability of recombinant cytokines and mAbs fostered the development of anti-cytokine Ab therapy to counteract deleterious cytokines in pathologic tissues of chronic diseases. This overview illustrates that this novel therapeutic approach can be achieved either by passive or active immunization. Heterologous or human murine chimeric mAbs of high neutralizing activity to a given cytokine, including TNFα, VEGF, IL-4, IL-5 and others, have been produced for passive Ab therapy. On the contrary, induction of neutralizing Abs by vaccine procedures implies a source of preexisting anticytokine B cells to synthesize and release them. Given the evidence for considerable censoring of self-reactive B cells, including clonal ignorance [67], clonal deletion [68] and clonal anergy [69], the common occurrence of effective autoAb response under physiological conditions seems paradoxical [70]. Nevertheless, vaccine procedures might trigger high titers of circulating autoAbs, neutralizing pathogenic self cytokines by breaking B cell tolerance, under certain conditions – namely: 1) in the presence of T cell help, supplied by a foreign carrier protein and; 2) following polyclonal B cell activation, as found in some pathologies, including AIDS [4], or experimentally after lipopolysaccharide or CpG stimulation [13,70]. Of interest, immunization that fulfils either condition does not trigger a specific anticytokine T cell activation, which, in fact, is not required for the objective of Ab therapy and is not beneficial. Autoreactive T cells, synergizing with specific B cell clones, would sustain and perpetuate high-affinity autoAb production and undesirable autoimmune disorders [70]. Also, the absence of autoreactive T cell help, characteristic of anti-cytokine immunization, is likely to account for the transient and non-permanent production of effective anti-cytokine autoAbs [26]. It should be emphasized, however, that, although not permanent, as in conventional vaccination (Table 1), these Abs can last for 3–6 months before declining, particularly when triggered by carrier-cytokine immunogens.

Anticytokine Ab immunization proved to be safe and effective, as initially shown by Sadick *et al.*, who blocked allergic reactions by anti-IL-4 Abs in *Leishmania major*-infected mice [29] and further confirmed by multiple investigations into passive and active anticytokine immunization; of which, the data from M. Feldmann's group, on anti-TNFα immune therapy in RA [22,39], is prominent. Notably, all of these investigations showed that the efficacy of both passive and active immunizing treatments to combat cytokine dysfunctions was ephemerous. Repeated booster injections were required to maintain circulating high Ab titers and durable therapeutic effects. Also, because these immunizations do not directly target the causative pathogens (cancer cells, viruses and other microbial infections or allergens), it might be beneficial to couple them with directed treatments, such as surgery, chemotherapy, antiviral treatments and vaccines, as demonstrated in cancer cases [40].

The main characteristics of these two types of Ab immunizations are compared in Table 1. Either of these immunizing procedures might be therapeutically applied to antagonize deleterious cytokines (see indication section). The selection of a particular procedure is guided by logistic considerations, such as the current availability of the vaccine products, the source and characteristics of the active principles (mAbs or kinoids), including the anticytokine neutralizing capacity they exhibit. In effect, their use could become competitive in some instances but, as in conventional vaccinations (vaccine versus serotherapy), are more often complementary. As an example, it can be speculated that, during the acute phase of Crohn's disease, effective mAbs might be administered to immediately stop the ulcerative crisis [22,39], wheras, simultaneous priming injections of TNFα kinoid could trigger high titer neutralizing Abs within a month. Also, bimonthly injections of heterologous or humanized chimeric Abs against a relevant pathogenic cytokine, as generally required to treat chronic diseases, could trigger an anti-idiotypic Ab response, resulting in disease relapse [22,32]. This complication can be overcome using a new generation of high-affinity Abs of human origin or by switching from passive to active Ab immunization.

In conclusion, the initial preclinical and clinical data have documented the feasibility, safety and efficacy of both immunizing procedures, and thus, we predict that, if appropriately prescribed and properly applied, active and passive immune therapy, targeting specific deleterious cytokines, will be expanded in the immediate future. It appears that this view is now shared by the pharmaceutical industry, which is currently investing in the development of these therapeutics (Table 2).

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