The impact of biological agents interfering with receptor/ligand binding in the immune system

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Abstract. – We herein discuss the impact of biological agents based on the ability of monoclonal antibodies to target specific molecules. This approach has given to clinical immunologists a spectrum of drugs able to manipulate the immune system.

In the first session, we discuss drugs targeting T-cell function by: (1) targeting CD28 mediated costimulation (Abatacept and Belatacept); (2) interfering with interleukin-2 receptor (Basiliximab and Daclizumab); (3) blocking cell adhesion and homing (Alefacept, Efalizumab, Natalizumab).

The second session is dedicated to drugs targeting cytokines or their receptors. The best known and largely experimented case is represented by drugs targeting tumor necrosis factor (TNF) (Infliximab, Adalimumab, Certolizumab) or its p75 receptor (Etanercept). However, newer products are now available to target other inflammatory cytokines including IL-6, IL-8, IL-12, IL-15, IL-18, IL-23.

These agents have the potential to become powerful tools in the control of several immune-mediated diseases, especially auto-immune and inflammatory ones. They translate into reality the prediction that antibodies will eventually become “magic bullets which seek their own target” (P. Ehrich, 1906).

Key Words: Biological immunosuppressors, T-lymphocytes, Cytokines, Immune-mediated diseases.

Introduction

Since the detection of human T-lymphocytes in the Seventies, our knowledge of basic and clinical immunology has dramatically increased, with the discovery of the basic mechanism of immune-recognition and the awareness that several diseases have an immune pathogenesis. Yet, the pharmacological repertoire available to the clinician has remained considerably poor and limited to aspecific immunosuppressors, such as corticosteroids, cyclophosphamide, azathioprine and mycophenolate mofetil.

The toxicity of available drugs has stimulated a great deal of research. In particular, the systematic study of products from bacteria and fungi has led to the development of a number of medicines, including the three immunosuppressive drugs: Cyclosporin A, FK506 or tacrolimus (a macrolide compound from the filamentous bacterium Streptomyces tsukubaensis) and Rapamycin (also known as sirolimus).

Cyclosporin A and tacrolimus block T-cell proliferation by inhibiting the phosphatase activity of a Ca^{2+}-activated enzyme called calcineurin at nanomolar concentrations. In addition, both drugs reduce the expression of several cytokine genes that are normally induced on T-cell activation, therefore this drug is now largely used in transplantation and autoimmune diseases\(^1,2\). The mechanism of action of cyclosporin A and tacrolimus is now fairly well understood\(^3\). Each binds to a different group of immunophilins: cyclosporin A to the cyclophilins, and tacrolimus to the FK-binding proteins (FKBP). These immunophilins are peptidyl-prolyl cis-trans isomerases but their isomerase activity does not seem to be relevant to the immunosuppressive activity of the drugs that bind them. Rather, the immunophilin: drug complexes bind and inhibit the Ca^{2+}-activated serine/threonine phosphatase calcineurin. Rapamycin (sirolimus), another Streptomyces macrolide, has a different mode of action from either cyclosporin A or tacrolimus. Like tacrolimus, it binds to the FKBP family of immunophilins. However, the rapamycin: immunophilin complex has no effect on calcineurin activity but, instead, blocks the interleukin-2 (IL-2) induced signal.

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transduction pathway that is an essential step of T-cell activation.

The limits of all these potent immunosuppressors are related to their lack of specificity, with related induction of generalized immune-suppression and potentially severe side effects. Therefore, the goal of immunosuppression remains that of inducing specific tolerance limited to the block of the immune response against a specific antigen (i.e., antigens triggering immune-rejection or autoimmune diseases).

Taking advantage from the availability of monoclonal antibodies, a new class of biological reagents has been developed which can target a single protein of the immune system. A variety of monoclonal antibodies-based treatments have been developed as anti-neoplastic drugs by targeting tumor-associated antigens. However, in this review, we will limit our discussion to those biological agents that interfere with the immune response. These agents may be divided in two main groups: (1) Drugs targeting T-lymphocytes functions and (2) Drugs targeting cytokines and their receptors.

**Drugs Targeting T-lymphocytes Functions**

The insights gained in the understanding of T-cell co-stimulation have provided a basis for the development of more selective drugs. In fact, T-cells are first triggered by the binding of their T-cell receptor (TCR) with the peptide, presented by antigen presenting cells (APC) in the context of HLA-Class I molecules, acting as a ligand for the CD8 on a subset of T-cells, or HLA-Class II, the ligand for the other main subset of T-lymphocytes cells. Binding is further stabilized by a number of additional receptor/ligand pathways such as adhesion molecules.

However, more important was the better understanding of the co-stimulatory pathway. In fact, antigen recognition will not lead to cell activation, but rather to programmed cell death (PCD) or apoptosis, unless a further co-stimulus is provided. This results as a consequence of the crosslinking of the CD28 T-cells molecule by CD80 and CD86 molecules expressed on APC. When triggering of the TCR is associated to co-stimulation, T-cell activation occurs resulting in expression of activation markers on the cell surface and eventually leading to cell proliferation. The feed-back regulation of immune activation is provided by a different molecule CD152 or cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), that is 20 times more avid then CD28 and delivers inhibitory signals. The possibility to block costimulation has the potential to achieve induction of selective tolerance. In fact, only the T-cells engaged by the specific antigens will succumb to apoptosis in the lack of appropriate costimulation.

**Co-stimulation block**

Based on these findings, in the Nineties, a human fusion protein combining the extracellular portion of CTLA-4 with the constant-region fragment (Fc) of human IgG1 (Abatcept) was developed and showed to be effective, alone or in combination with other immunosuppressors, in several clinical conditions, such as rheumatoid arthritis, sarcoidosis and psoriasis, but had little effect in transplantation. Later, a modified version of Abatacept was developed by substitution of 2 aminoacids, this leading to a new agent (Belatacept or LEA29Y) with increased avidity for the ligand. Belatacept has now been used in a variety of clinical conditions. In patients with active rheumatoid arthritis and an inadequate response to at least three months of anti-tumor necrosis factor-alpha (TNF-alpha) therapy, Abatacept produced significant clinical and functional benefits. Encouraging results were also obtained in metastatic melanoma. This drug also showed effects in human transplants: in a randomized study in renal transplants recipients showed similar efficacy with less nephrotoxicity as compared to cyclosporin and may preserve the glomerular filtration rate and reduce the rate of chronic allograft nephropathy.

The benefits of a co-stimulation block potentially includes virtually all autoimmune diseases as well as reversal of hyperresponsiveness in infectious diseases. More recently an additional anti-CD80 reagent (Galiximab or IDEC-114) has became available. It was also shown to be effective in renal allo-
trasplant\textsuperscript{13} and psoriasis\textsuperscript{14,15}. Since CD80 is also expressed in neoplastic lymphoid cells, this antibody also has activity in lymphoproliferative diseases as several antibodies directed against B-cell markers \textsuperscript{16-17}. 

**Interleukin-2 Receptor Block**

Upon activation, T-cells produce several cytokines and express their receptors. Interaction of IL-2 and its receptor represent the most important signal in further proliferation of the antigen-specific clone. The IL-2 receptor is formed by three chains. The beta and gamma chains are expressed constitutively and they bind IL-2 with moderate affinity. Activation of T-cells induces the synthesis of the alpha chain (CD25) and the formation of the high affinity heterotrimeric receptor. It was proposed that a selective immunosuppression of activated cells can be obtained blocking the expression of CD25\textsuperscript{18}. Recently, two monoclonal antibody preparations against the alpha chain of the IL-2 receptor became available for use, Basiliximab and Daclizumab, a chimeric and a humanized antibody, respectively. A meta-analysis in more than 8,000 patients showed that adding anti-interleukin-2 receptor antibodies to cyclosporin based immunosuppression reduces episodes of acute rejection at six months by 49%. There is no evidence of an increased risk of infective complications\textsuperscript{19}. Suppression was also reported in other models of transplantation: in kidney-pancreas transplant\textsuperscript{20}, as well as liver and cardiac ones\textsuperscript{21-22}. However, in cardiac transplantation, while the rate of rejection was lower in the Daclizumab group, significant side effects were observed when cytotoxic treatment was associated\textsuperscript{23-24}. The two anti-CD25 preparations have been shown to possess virtually identical effects and for this reason are considered to be equivalent even in the lack of a trial directly comparing the two antibodies\textsuperscript{25}.

**Blocking Adhesion or Homing Molecules**

In order to stabilize the T-cell/APC interactions, others receptor/ligand systems have been described and can now be targeted by new biological agents.Efalizumab binds to the alpha subunit (CD11) of LFA-1 and prevents LFA-1 binding to intracellular adhesion molecule (ICAM)-1 and has shown effects in psoriasis\textsuperscript{26-28}.

Alefacept is a human LFA-3/IgG1 fusion protein and is under clinical investigation for the treatment of psoriasis and rheumatoid arthritis. The LFA-3 portion of Alefacept binds to CD2 receptors on T cells to block the natural interaction between LFA-3 (CD58) on antigen-presenting cells and CD2 on T cells, thus inhibiting the CD2-mediated activation pathway\textsuperscript{29-30}.

Finally, promising results have been achieved with a new biological agent named Natalizumab\textsuperscript{31}, a humanized antibody directed against the alpha4-beta7 integrin, that is primarily involved in the recruitment of lymphocytes to the gut\textsuperscript{32}. This drug has been shown to give promising results in ulcerative colitis\textsuperscript{33}, even if these data must be considered as preliminary since this study, performed in 181 patients, was limited to a short term observation.

**Drugs Targeting Cytokines and Their Receptors**

Cytokines are proteins made by cells that affect the behavior of other cells, thus assuming a central role in the development of immune responses. Recombinant cytokines such as alpha, beta and gamma Interferon (INF) have been widely used in the last 20 years. For instance, alpha IFN has the ability to up-regulate HLA antigens thus increasing the ability of cytotoxic T-cells to kill their targets and is now extensively used in viral infections such as hepatitis C virus (HCV) infection\textsuperscript{34}. Gamma interferon is used in pulmonary fibrosis\textsuperscript{35}. Interferon-beta is used in the treatment of multiple sclerosis where increases IL-10 and inhibits IL-12, a critical cytokine in the pathogenesis of the disease\textsuperscript{36}. IL-2 is another potent immunostimulant which ameliorates the immuno-responses in neoplasias (such as melanoma\textsuperscript{37} and leukemia\textsuperscript{38}) or viral diseases\textsuperscript{39}.

A more recent approach has been used exploiting the use of monoclonal antibodies directed against the cytokines or their receptors.
to block their activity. Drugs against the IL-2R alpha are an example of this group of new agents, but since they affect primarily T-cell function, have been more appropriately discussed above in section 2.

**Blocking TNF**

TNF-alpha (OMIM *191160) is produced by macrophages, natural-killer (NK) cells and T-lymphocytes. It is a multifunctional proinflammatory cytokine, with effects on lipid metabolism, coagulation, insulin resistance, and endothelial function. It has been shown to contribute to the pathogenesis of inflammation in several diseases including rheumatoid arthritis, Crohn’s disease (CD) and psoriasis upon interaction with the p75 TNF receptor (CD120b). Because of its severe effects, a great effort has been placed to neutralize its activity, resulting in the first two registered drugs targeting a cytokine and its receptor (Infliximab and Etanercept). These biological agents are now well established in clinical use.

**Infliximab**

Infliximab is a chimeric IgG1kappa monoclonal antibody. It is composed of human constant and murine variable regions and binds specifically to human TNF-alpha. Infliximab blocks TNF in the serum and at the cell surface and lyses TNF-producing macrophages and T cells through complement fixation and antibody-dependent cytotoxicity. By blocking the action of TNF-alpha, Infliximab reduces the signs and symptoms of inflammation.

Infliximab was first approved in the United States for the treatment of Crohn’s disease in 1998, in patients with moderate to severe CD and also in patients who have a type of CD in which fistulas form. The successful use has been well documented in several large clinical trials. Infliximab is approved for use alone or combined with methotrexate for treating moderate to severe rheumatoid arthritis. It also is approved for the treatment of active psoriatic arthritis. Infliximab has also been reported to be helpful in reducing the joint inflammation of juvenile rheumatoid arthritis, uveitis, and for sarcoidosis that is not responding to traditional therapies.

Van den Bosch et al. examined the efficacy of the Infliximab versus placebo in the treatment of spondylarthropies. Robust improvements from baseline were seen in laboratory measures of disease activity, night pain scores, numbers of tender and swollen joints and other measures of axial and peripheral disease.

Infliximab is an effective treatment for moderate-to-severe psoriasis. When a total of 378 patients were randomized to receive Infliximab or a placebo, after 10 weeks, 80% of the patients receiving Infliximab experienced improvements of at least 75% in their psoriasis compared to 3% of the patients receiving placebo. This finding remained statistically significant at week 24, with 82% of patients receiving Infliximab experiencing improvements of at least 75% compared to 4% of patients in the placebo group. Complete clearing of the skin was observed in 26% of patients receiving Infliximab compared to no patients receiving placebo. The numbers of adverse events were similar in both treatment groups. The benefits of Infliximab were generally well-maintained throughout the one year trial. However, Infliximab may induce side effects such as, chest pain, nausea, fever, facial flushing, headache, rash, difficulty breathing, low blood pressure, fatigue, dizziness, and possibly risk of developing lymphoma (NHS guidance, 2005).

Infliximab decreases the activity of immune system, thus increasing the risk of infections. A small number of patients may develop an infection that requires antibiotics and hospitalization. Infliximab can reactivate tuberculosis (TB) in people who have been previously infected with TB. Before starting Infliximab treatment, patients should be screened with a tuberculin skin test and a chest X-ray. If the skin test is positive or the
chest X-ray suggests previous exposure to TB, it will need treatment to prevent TB reactivation.

Its chimeric composition (human constant and murine variable regions, with approximately 25% of antibody sequence of murine origin) has been associated with problems of immunogenicity, which may lead to infusion reactions and loss of efficacy. The development of antibodies against Infliximab is associated with an increased risk of infusion reactions and a reduced duration of response to treatment. Infliximab becomes less effective as the concentration of antibodies increases. However, taking a medication to suppress the immune system (such as azathioprine, 6-mercaptopurine, or methotrexate) along with Infliximab reduces the risk of reaction and increases Infliximab’s effectiveness. The development of antinuclear (ANA) and anti-double-stranded DNA (dsDNA) antibodies has been described in CD and rheumatoid arthritis (RA) trials. In particular, according to the reported safety data, 63.8% of RA patients and 49.1% of CD patients developed newly positive ANA during Infliximab treatment, and 13% and 21.5%, respectively, developed newly positive anti-dsDNA antibodies. Rare cases of lupus-like syndrome at the beginning Infliximab treatment for CD were also reported.

Vermeire et al. described a cumulative ANA incidence at 24 months in 71 of 125 patients (56.8%). Almost half of these patients developed ANA after the first infusion, and > 75% became ANA-positive after fewer than three infusions. However, only two patients (both antihistone and dsDNA-positive) developed drug-induced lupus without major organ damage, and one developed autoimmune haemolytic anaemia. Decreased white and red blood cell and decreased platelet counts have been reported with Infliximab.

**Etanercept**

Etanercept is a synthetic dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) TNF receptor (TNFR) linked to the Fc portion of human IgG1. The Fc component of etanercept contains the C\v2 domain, the C\v3 domain and hinge region, but not the C\v1 domain of IgG1.

Etanercept is indicated for reducing signs and symptoms, inducing major clinical response, inhibiting the progression of structural damage, and improving physical function in patients with moderately to severely active rheumatoid arthritis. It can be initiated in combination with methotrexate or used alone, most patients receiving benefit within 3 months.

Etanercept, administered twice weekly, is active in children with active Juvenile Rheumatoid Arthritis in at least five joints, not responded adequately to methotrexate or who have been unable to tolerate treatment with.

Etanercept improves physical function in patients with psoriatic arthritis. It can be used in combination with methotrexate in patients who do not respond adequately to methotrexate alone. It was shown to be effective in about 50% of psoriatic arthritis patients who used it. Clinical responses were apparent at the time of the first visit (4 weeks) and were maintained through 6 months of therapy.

Gorman et al. found in 40 patients that a four month course of etanercept resulted in a significant and sustained improvement in patients with ankylosing spondylitis. Clinical improvements were quickly observable and are sustained over time.

Etanercept is indicated for the treatment of adult patients with chronic moderate to severe plaque psoriasis who are candidates for systemic therapy or phototherapy. The treatment of psoriasis with etanercept led to a significant reduction in the severity of disease over a period of 24 weeks.

Etanercept is not recommended in patients with preexisting disease of the central nervous system (brain and/or spinal cord) or for those with multiple sclerosis, myelitis, or optic neuritis.

The most common side effects are mild to moderate itching, pain, swelling and redness at the site of injection. Headache, dizziness, nasal and throat irritation also occur. TNF alpha has an important role in the responses of the immune system to infections. Thus, as for infliximab, blocking the action of TNF alpha with etanercept may worsen or increase the occurrence of infections, and patients with serious infections should not receive etanercept. Moreover, etanercept should be discon-
tained if a patient develops a serious infection. Etanercept should also be used with caution in patients prone to infection, such as those with advanced or poorly controlled diabetes. Finally, rare cases of seriously pancytopenia have been reported in patients using etanercept. Since etanercept is a relatively new drug, there is limited information on long-term risks.

*Other anti-TNF biologic agents*

Although Infliximab has clear-cut positive effects, its activity is limited by the insur-gence of anti-idiotypic antibodies. Therefore the quest for new anti-TNF drugs has continued. The process of “humanisation” of monoclonal antibodies entails transfer of the antigen binding regions, (complementarity determining regions or CDR), of the murine variable domain to a human antibody. The resulting humanised monoclonal antibody is approximately 95% human protein and is relatively less immunogenic than chimeric antibodies.

A humanised monoclonal antibody to human TNF-alpha (CDP571) was constructed by linking the CDR of a murine antihuman TNF monoclonal antibody to a human IgG4 antibody. Three phase II trials suggested that intravenous CDP571 may be well tolerated and effective for the short term treatment of mild to moderate Crohn's disease and steroid dependent Crohn's disease.43,59,60.

CDP571 is modestly effective for short but not long term treatment of unselected patients with moderate to severe CD. The reasons of this limited effect are unclear. The frequency of anti-idiotypic antibodies to CDP571 (10.9%) compares favourably with results that have been reported for Infliximab, where as many as 60% of retreated patients have developed antibodies.41,47,48.

Certolizumab (CDP-870) is a new agent that employs a novel strategy to neutralise TNF-alpha. CDP870 comprises a Fab' fragment of a humanized monoclonal antibody that is a potent neutralizer of TNF-alpha. The Fab' fragment retains high affinity and potency, but lacks the Fc portion of the parent IgG4 antibody. The site-specific addition of two molecules of polyethylene glycol to the antibody fragment increases its plasma half-life to approximately 2 weeks, which reduces the required frequency of dosing. Subcuta-neous administration of CDP870 has been associated with marked clinical efficacy and good tolerability in patients with moderate-to-severe CD and rheumatoid arthritis. Although intended for subcutaneous administration, CDP870 may also be effective for the treatment of CD when administered intravenously. By administering CDP870 intravenously, it was possible to explore the use of higher doses than it was practical to deliver subcutaneously.59.

Adalimumab is a human-derived recombinant IgG1 monoclonal antibody engineered by gene technology, that binds to TNF-alpha but not TNF-beta. This antibody has been extensively studied in vitro as well as in vivo, it is approved for reducing the signs and symptoms, inducing major clinical response, slowing the progression of joint damage, and improving physical function in adult patients with moderate to severe rheumatoid arthritis. It is also approved for reducing the signs and symptoms of active arthritis in patients with psoriatic arthritis. It is also used for therapy-resistant sarcoidosis.61,62.

*Blocking Other Cytokines*

Blocking of TNF has been only the first of the strategy to develop drugs targeting cytokines, including IL-6, IL-8, IL-12, IL-15, IL-18 and IL-23.

**IL-6**

IL-6 (OMIM *147620), is produced by T-cells, macrophages and endothelial cells. It has pro-inflammatory activity. Targeting of IL-6 has the potential to be useful in certain neoplastic and autoimmune diseases.63,64.

**IL-8**

IL-8 (OMIM*146930) is a pro-inflammatory cytokine acting through the activation of macrophages. Approaches are being made to target IL-8.65.

**IL-12**

Interleukin-12 (OMIM*161560) (formerly NKSF, for natural killer cell stimulatory factor, or CLMF, for cytotoxic lymphocyte maturation factor) is a novel cytokine cloned from B-cell lines acting on both T and NK-cells by inducing inflammation. It is produced primar-
ily by inflammatory macrophages, and both macrophages and microglia. Anti-IL-12 antibodies may have a role in the treatment of Crohn's disease.66,67.

**IL-15**

Interleukin-15 (OMIM*600554) is a cytokine believed to maintain the memory T-cell pool. It has been suggested that the inflammatory cytokine IL-15 plays an important role in the development of several autoimmune diseases. Experimental models have shown that an antagonist mutant IL-15/Fc protein has positive effects in delayed-type hypersensitivity arthritis and allograft rejection.68,69.

**IL-18**

IL-18 (OMIM*604113) is a pleiotropic cytokine whose excessive production may contribute to chronic inflammatory conditions. Anti IL-18 antibodies are being experienced in experimental models.70,71.

**IL-23**

IL-12-related cytokine IL-23 (OMIM*161560) is composed of the IL-12 p40 subunit and the p19 subunit. IL-23 mediates late-stage inflammation and seems to be necessary for chronic inflammation and may have a role in the treatment of multiple sclerosis.72,73.

Finally, an even different approach, i.e., targeting human immunoglobulins with a humanized monoclonal Anti-IgE antibody as been proposed for the treatment of allergic diseases (Omalizumab).74.

**Conclusion**

In summary, we have shortly reviewed some of the biological agents based on monoclonal antibodies specificity that are promising for manipulating the immune response. Such area of research has a powerful rationale and will show in the immediate future its capability to provide new tools for treating diseases in areas such as internal medicine, immunology, gastroenterology, reumathology, dermatology, allergy and oncology where antineoplastic antibodies are developed on a weekly basis. It will also considerably affect the market bringing the need for additional resources in the treatment of diseases. This approach is fulfilled prediction the prophecy made by the 1908 Nobel prize winner for the discovery of antibodies: “some substances... would represent... magic bullets which seek their targets of their own accord” (Paul Ehrlich, 1906).

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