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Monoclonal antibodies and fusion proteins in medicine

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Humanized antibodies and decoy receptors have been introduced in clinical practice to treat malignancies and systemic autoimmune disease because they ablate specific cells or disrupt pathogenic processes at distinct points. Reported clinical responses offer hope to treatment-resistant patients, particularly those with lymphomas and rheumatic diseases. Side effects from the use of biologic agents include lymphokine release syndrome, reactivation of tuberculosis, and immunosuppression. Further insights are needed regarding limitation of adverse effects, correct use in conjunction with existing drugs, and treatment of patients in whom resistance develops. (*J Allergy Clin Immunol* 2005;116:721-9.)

Key words: *Cancer, lymphoma, transplantation, rheumatoid arthritis, systemic lupus erythematosus, psoriasis*

Recent improvements in the understanding of cellular and molecular pathogenesis of immune diseases have led to the advent of novel rational therapeutics designed to disrupt specific pathogenic processes. Advances in molecular technology have facilitated the construction and production of biologic agents, such as humanized mAbs and decoy receptors, which disrupt the action of cytokines on cells, as well as cell-cell interaction, and also deplete desired target cells.

The side effects caused by the injection of xenogeneic, usually murine, mAbs have been significantly limited with the development of chimeric, humanized, and finally entirely human mAbs able to bind desired human target molecules. The methods used to engineer humanized and human mAbs and their industrial production are beyond the scope of this review, but in short, an entirely antigenic

Abbreviations used

ADCC: Antibody-dependent cell-mediated cytotoxicity
CD: Crohn's disease
CD40L: CD40 ligand
CTLA4: Cytotoxic T lymphocyte-associated antigen 4
EGF: Epidermal growth factor
EGFR: Epidermal growth factor receptor
FDA: US Food and Drug Administration
FP: Fusion protein
HL: Hodgkin's lymphoma
LFA: Lymphocyte function-associated antigen
NHL: Non-Hodgkin's lymphoma
RA: Rheumatoid arthritis
RIT: Radioimmunotherapy
SLE: Systemic lupus erythematosus
VEGF: Vascular endothelial growth factor
VEGFR: Vascular endothelial growth factor receptor

nonhuman protein, such as a murine mAb, becomes "human friendly" when small parts of the initial murine mAb are "engrafted" or inserted onto human immunoglobulin molecules, creating either chimeric (only the Fc part of the immunoglobulin molecule is human) or humanized (only the complementarity-determining regions of the novel immunoglobulin molecule are murine and 90% to 95% of the molecule is human) mAbs.

Alternative to the engineering of humanized mAbs is the construction of fusion proteins (FPs), the fusion of the Fc part of human IgG1 with a natural human soluble receptor or ligand of a target molecule. FPs are artificial yet completely human molecules that retain the function of the soluble receptor or ligand, but with the addition of the Fc part of IgG1, they acquire sufficient biologic half-life to make them clinically useful. The number of mAbs and FPs used in the clinic is steadily increasing, and in this report we will review the most commonly used (Table I).

TRANSPLANTATION

The murine anti-human CD3 mAb (OKT3) was the first mAb introduced in clinical medicine and was used to

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TABLE I. Humanized antibodies and FPs in clinical trials or introduced into clinical practice

Name	Target antigen-molecule	Application
Antibodies		
OKT3	CD3	Renal transplants
Basiliximab (chimeric)	IL-2 receptor α -chain	Organ transplants
Daclizumab (humanized)	IL-2 receptor α -chain	Organ transplants, noninfectious uveitis
Paviluzumab	Respiratory syncytial virus	Infants with bronchopulmonary dysplasia
Trastuzumab	Receptor tyrosine kinase ERBB2	Cancer
Cetuximab	Receptor tyrosine kinase EGFR	Cancer
Bevacizumab	VEGFR1 and VEGFR2	Cancer (metastatic)
Rituximab	CD20	B-cell lymphomas, autoimmunity
Ibritumomab (yttrium 90 labeled)	CD20	B-cell lymphomas
Tositumomab (iodine 131 labeled)	CD20	B-cell lymphomas
Alemtuzumab	CD52	Hematopoietic malignancies
Epratuzumab	CD22	B-cell lymphomas
Infliximab	TNF- α	RA, CD
Adalimumab	TNF- α	RA, CD
MRA	IL-6 receptor	RA
Anti-IL-2	IL-2	RA
Efalizumab	CD11a	Psoriasis
IDEC-131	CD40L	SLE
Ruplizumab	CD40L	SLE
Omalizumab	IgE	Asthma
FPs		
Etanercept	TNF receptor (p75)	RA
Abatacept	CTLA4	RA, psoriasis

deplete T cells in patients with acute rejection of renal allotransplants.¹ Cross-linking of CD3 with OKT3 initially activates T cells before activating complement and lysing the targeted T cells. The use of OKT3 is associated with increased susceptibility to infections and the so-called cytokine release syndrome. Cytokine release syndrome is characterized by shaking chills, fever, arthralgia, diarrhea and vomiting, hypotension, and even the development of respiratory distress as a result of pulmonary infiltrates caused by capillary leakage. Because OKT3 is a xenogenic protein, it is recognized by the immune system as an antigen, induces the production of anti-murine antibodies, and is cleared rapidly from the circulation. The above side effects limit the repeated use of OKT3 and have encouraged the development of HuM291, a humanized OKT3. In a phase I trial a single dose of HuM291 rapidly depleted circulating T cells in a dose-dependent manner and was

associated with only mild-to-moderate symptoms related to cytokine release.²

Acute graft rejection is a T cell-mediated immune response and depends on the presence of IL-2. IL-2 binds to its high-affinity T-cell surface receptor (IL-2 receptor) α -chain, which is expressed on the surface of activated T cells. Two mAbs that do not kill T cells but block the IL-2 receptor α -chain and are currently used in patients receiving transplants are the chimeric mAb basiliximab and the humanized mAb daclizumab. The clinical effectiveness of both these mAbs in preventing acute renal rejection when used in conjunction with conventional antigrraft rejection treatment has been documented. Daclizumab decreased the incidence of rejection from 43% to 28%, and basiliximab (in 2 studies) decreased the incidence of rejection from approximately 45% to 32%.³⁻⁶ Long-term efficacy and the retention and viability of the allografts have not been fully evaluated.

UVEITIS

Daclizumab was recently reported to hold promise for the treatment of noninfectious uveitis. No serious side effects were recorded in an open and nonrandomized feasibility study, and the need for concomitant use of immunosuppressive therapy was diminished.⁷

INFECTION AND SEPSIS

TNF- α has been detected in increased amounts in patients with sepsis, and it is considered to contribute significantly to sepsis-associated pathology. Therefore anti-TNF- α treatment has been studied in a trial, but this was soon interrupted because of increased mortality.⁸

The humanized anti-respiratory syncytial virus mAb paviluzumab is used in the treatment of term and preterm infants with bronchopulmonary dysplasia for the prevention of infection with respiratory syncytial virus. The use of paviluzumab leads to a 50% reduction in the incidence of episodes of infection with this virus.⁹

MALIGNANCIES

Solid tumors

Cancer cells express antigens on their surface, and if these molecules are underexpressed or not expressed on the surface of normal cells, then they represent candidate targets for therapeutic mAb. Trastuzumab is the first mAb approved by the US Food and Drug Administration (FDA) for the treatment of solid tumors. It is a humanized IgG1 mAb that targets the receptor tyrosine kinase ERBB2 (HER2) and blocks its action. It is expressed on the surface membrane of tumor cells from 30% of patients with breast cancer and signifies poor prognosis. Trastuzumab is currently in clinical use for the treatment of HER2⁺ metastatic breast cancer and is more effective in patients whose cancer cells express increased amounts of

ERBB2.¹⁰ The clinical efficacy of trastuzumab, used either alone or in conjunction with standard chemotherapy, has been documented in various studies. The addition of trastuzumab might improve the response rate by 15% and extend survival by 5 months.¹¹⁻¹⁵ Although trastuzumab infusion is rarely associated with significant side effects, its use has been associated with cardiotoxicity, which increases when administered in combination with the cardiotoxic anthracyclines.¹⁶ Therefore cardiac function should be evaluated before and during treatment.

Cetuximab is a chimeric IgG1 mAb that binds to the receptor tyrosine kinase epidermal growth factor receptor (EGFR) (ERBB1, HER1), which is expressed on the surface of various epithelial cancer cells, and interferes with the binding of its ligand, epidermal growth factor (EGF). Cetuximab-mediated inhibition of ligand binding leads to arrest of cancer cell growth and apoptosis. Cetuximab has been approved for the treatment of EGFR⁺ metastatic colon cancer, and when added to standard chemotherapy, it increases the response rate from 11% to 23% and extends survival by 1.7 months.¹⁷ The addition of cetuximab to standard treatment protocols in patients with non-small cell lung cancer, head and neck cancer, and pancreatic cancer has produced encouraging results.¹⁸⁻²⁰ Cetuximab administration is associated with an acne-like skin rash, which predicts better treatment outcome.²¹

Bevacizumab is a humanized mAb directed against the vascular endothelial growth factor (VEGF) receptors type 1 and 2 (VEGFR1 and VEGFR2). VEGF is a growth factor that stimulates angiogenesis. Blocking VEGFR1 and VEGFR2 inhibits the formation of neoplastic capillaries and promotes apoptosis of vascular endothelial cells. The use of bevacizumab in metastatic colorectal cancer, with or without standard chemotherapeutic agents, leads to better response rates and prolongs survival by several months.²² Trials of bevacizumab administration in patients with metastatic breast cancer, clear-cell renal cancer,²³ and non-small cell lung cancer have also produced encouraging results.

Hematologic neoplasms

The chimeric IgG1 anti-human CD20 mAb rituximab was the first mAb approved by the FDA for the treatment of non-Hodgkin's lymphoma (NHL). Lymphomas represent the fourth most commonly diagnosed neoplasm, and their incidence is increasing. Rituximab targets the transmembrane protein CD20, which is present on the surface of normal pre-B and mature B cells. CD20 is also expressed on the surface membrane of malignant B cells found in low-grade and follicular NHL and the neoplastic B cells found in the lymphocyte-predominant subtype of Hodgkin's lymphoma (HL). Rituximab kills malignant CD20⁺ cells either through complement-dependent or antibody-dependent (Fc receptor-mediated) cell-mediated cytotoxicity. In addition, pretreatment of neoplastic cells of B-cell origin with rituximab sensitizes them to chemotherapy.²⁴ The follicular type is the most common subtype of NHL (30%), and although indolent clinically,

it represents an incurable disease. Administration of rituximab as a single agent induces a 1-year remission in 50% of patients.^{25,26} The use of rituximab in conjunction with standard chemotherapy results in an impressive 95% response rate.²⁷⁻²⁹ In patients with high-grade lymphomas, administration of rituximab alone resulted in clinical response in 32%, whereas when used in conjunction with chemotherapy, it resulted in clinical response in 76% of patients.^{30,31} In patients with lymphocyte-predominant HL (a good-prognosis subset of patients with HL), rituximab was used to treat relapses, and it produced response rates of 86% to 100%.³²

Rituximab has been used successfully in patients with Waldenström's macroglobulinemia,³³ posttransplantation lymphoma, and multiple myeloma.^{34,35} Unfortunately, rituximab caused increased rates of sepsis when given to patients with HIV-associated NHL.³⁶ The spectrum of diseases in which rituximab might offer a therapeutic advantage also includes immune hemolytic anemia, refractory immune thrombocytopenic purpura, and pure red cell aplasia.³⁷⁻³⁹

Although many patients respond initially to rituximab, they eventually become resistant or unresponsive. Inaccessibility of malignant cells and downregulation of surface CD20 expression might be responsible for the development of rituximab resistance. To overcome the problem of rituximab resistance, anti-CD20 mAb has been conjugated to radioactive elements (yttrium 90 and iodine 131) and has been used in the treatment of CD20⁺ rituximab-refractory or relapsed follicular low-grade NHL. This novel approach is termed radioimmunotherapy (RIT). Two different RIT agents, ibritumomab and tositumomab, both murine anti-human CD20 mAbs conjugated with yttrium 90 and iodine 131, respectively, have been approved by the FDA for use in rituximab-resistant lymphomas, producing impressive results.⁴⁰⁻⁴³ RIT agents kill target cells, as well as both innocent and guilty (neoplastic but mAb-inaccessible) bystander cells. Therefore their use is limited to patients having less than 25% bone marrow infiltration and safe numbers of neutrophils and platelets.

Another mAb used in hematologic malignancies is the humanized anti-CD52 mAb alemtuzumab (Campath-1H). Alemtuzumab kills malignant and normal hematopoietic cells bearing the cell-surface marker CD52. CD52 is present on the surface membrane of T and B cells, macrophages, monocytes, and natural killer cells. Alemtuzumab has been approved for use in refractory B-cell chronic lymphocytic leukemia, in which it induces a clinical response in one third of patients.⁴⁴ An 87% response rate has been reported when it is used in previously untreated patients with B-cell chronic lymphocytic leukemia.⁴⁵ The use of alemtuzumab has been associated with infusion reactions (a cytokine release syndrome that is preventable with the use of steroids) and with a significant rate of serious infections because it causes profound immunosuppression. It has been used successfully in patients with T-cell prolymphocytic leukemia and to prevent graft-versus-host disease.^{46,47}

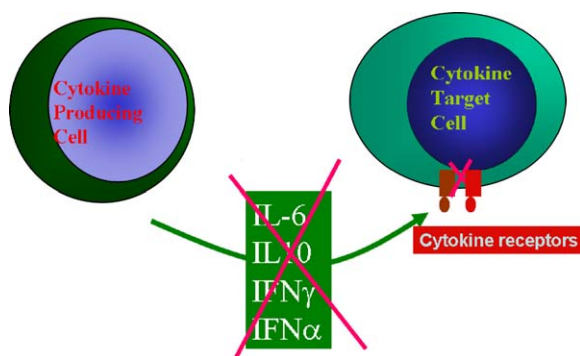


FIG 1. Biologic agents developed to disrupt lymphokine action. Immune cells produce cytokines that modulate the function of immune and nonimmune cells after binding to specific receptors. Humanized anti-cytokine mAbs form complexes with the respective cytokines and prevent their interaction with cell receptors. Decoy receptors bind circulating cytokines and prevent their interaction with cell-surface receptors.

Epratuzumab, a humanized IgG1 anti-human CD22 mAb, has also been introduced in the treatment of lymphomas.⁴⁸ CD22 is a cell-surface marker of B cells and represents an inhibitory B-cell surface receptor. One trial using a macaque-human chimeric anti-CD80 (a costimulatory molecule) mAb as a single agent in refractory lymphoma yielded a 15% response rate.⁴⁹ The CD30 molecule expressed on the surface of Reed-Sternberg cells in HL and on the cells of anaplastic large-cell NHL has been targeted by a variety of anti-CD30 mAbs.^{50,51}

MONOCLONAL ANTIBODIES AND FPs IN THE TREATMENT OF AUTOIMMUNE DISEASES

Anti-cytokine mAbs

Anti-TNF- α . It has been proposed that the proinflammatory cytokine TNF- α plays a key role in certain autoimmune diseases, such as Crohn's disease (CD)⁵² and rheumatoid arthritis (RA).⁵³⁻⁵⁵ The approaches used to block the action of TNF- α , as well as the activities of other cytokines, are depicted in Fig 1.

Infliximab, a chimeric anti-TNF- α mAb, was first approved for the treatment of CD, a disease in which TNF- α is overexpressed at sites of intestinal mucosal lesions. Treatment of patients with CD with infliximab results in remarkable clinical benefit. In a placebo-controlled study 65% of patients with CD receiving infliximab responded compared with 17% of those treated with placebo.⁵⁶ More importantly, a significant reduction in fistulae formation and a reduction in the "time to response" were noted in patients receiving infliximab.⁵⁷ Early response (ie, a clinical response after the third infusion) predicts patients who will benefit the most by continuing treatment for 1 year.⁵⁸ Patients with ulcerative colitis have also been treated with infliximab in clinical trials.⁵⁹ The results are favorable, and it is expected to be approved for the treatment of this disease as well.

The therapeutic efficacy of infliximab in patients with RA has been established in several clinical trials. Initially,

it was reported that the addition of infliximab to patients receiving methotrexate and experiencing only an incomplete response resulted in significant clinical improvement sustained for 1 year.⁶⁰ More importantly, radiographic progression of the disease was halted, regardless of the clinical response to infliximab.⁶¹ The clinical response occurs quickly, and if the patient shows poor response, the dose or the frequency of administration can be increased. The clinical and radiographic efficacy of infliximab in patients with RA is retained after the completion of a 2-year treatment.⁶²

An alternative approach to the use of humanized anti-TNF- α antibody is the use of etanercept, an FP consisting of the high-affinity type 2 receptor p75 fused with the Fc fragment of the human IgG1 molecule. This entirely human protein binds not only TNF- α but also TNF- β (lymphotoxin), interfering thus with the binding of both cytokines to their relevant cell-surface receptors. Etanercept has been proved in double-blind trials to be highly effective in the treatment of patients with RA⁶³ and is apparently superior to methotrexate both in terms of clinical and radiographic response.⁶⁴ Its effect is retained for at least 2 years.⁶⁵ The combination of etanercept and methotrexate results in even higher response rates.⁶⁶

Adalimumab, a fully human IgG1 mAb, has also been approved for the treatment of patients with RA. Adalimumab binds strongly to soluble and cell membrane-bound TNF- α . The clinical efficacy of adalimumab used in combination with methotrexate has been established in large clinical trials,⁶⁷ with a profound decrease in the clinical and radiographic reduction of the disease.⁶⁸ Anti-adalimumab antibodies are detected in patients treated with the mAb alone (without methotrexate).

TNF- α is expressed on the surface of TNF- α -producing cells before it is cleaved and released into the extracellular fluid. It binds onto 2 different types of receptors, type 1 (p55) and the high-affinity type 2 (p75). Both types of TNF- α receptors are also cleaved from the cell surface and can be found in a soluble form presumably counteracting the effects of their soluble ligand. Infliximab efficiently binds soluble forms of TNF- α , diminishing its biologic actions, but similarly binds to T cells expressing TNF- α on the surface and might kill the target cells through complement-mediated and antibody-dependent cytotoxicity. Killing of T cells expressing TNF- α on the surface membrane is thought to be responsible for the reactivation of tuberculosis and histoplasmosis in patients receiving anti-TNF- α mAb. Currently, patients scheduled to receive anti-TNF- α mAb treatment are subject to tuberculosis screening to limit reactivation.⁶⁹ Another side effect of anti-TNF- α treatment is the development of lymphomas.⁷⁰ Currently, this issue has not been adequately addressed, partially because RA itself represents a risk for the development of lymphoma.

Anti-TNF- α mAb treatment has been explored with encouraging results in patients with seronegative spondyloarthropathies, such as ankylosing spondylitis and psoriatic arthritis.⁷¹⁻⁷⁵ Etanercept is also effective in the

treatment of psoriasis and psoriatic arthritis, with significant results at the clinical and radiographic levels.⁷⁶⁻⁷⁸

Anti-IL-6 and anti-IL-6 receptor mAbs. IL-6 is a pleiotropic cytokine that is overproduced in patients with RA. Circulating and synovial fluid levels of IL-6 are increased in patients with RA, with the levels correlating with disease activity and extent of joint erosion.⁷⁹ Interference with the action of IL-6 through its receptor has been attempted with the use of a humanized IgG1 anti-human IL-6 receptor mAb called MRA. This mAb blocks the binding of IL-6 to IL-6 receptor, and its therapeutic efficacy was assessed in a randomized, double-blind, placebo-controlled trial of patients with active and refractory-to-treatment RA. The clinical response among these patients was high, with an absence of important side effects.⁸⁰

Anti-IL-12. IL-12 is responsible for the induction of a T_H1 cytokine response. An mAb directed against IL-12 was tested in patients with CD, and the recorded clinical response was as high as 75% compared with 25% in the control group. As expected, besides IL-12, the levels of IFN- γ and TNF- α decreased significantly in the group of patients treated with anti-IL-12 mAb.⁸¹

Immune cell-depleting mAb treatment: Anti-CD20 mAb in the treatment of RA and systemic lupus erythematosus

The role of B cells in RA pathogenesis has not been fully examined. On the basis of the encouraging results of an open-label study, the role of rituximab-mediated B-cell depletion was addressed in a double-blind, placebo-controlled trial including patients with RA resistant to treatment with methotrexate. Patients received 2 infusions of rituximab, either alone or with methotrexate or cyclophosphamide, and were followed for 1 year. The presence of rituximab resulted in significant clinical improvement without any notable side effects.⁸²

B cells in systemic lupus erythematosus (SLE) display abnormal signaling, express aberrant cell-surface markers (eg, CD40 ligand [CD40L]), and finally produce autoantibody and probably present autoantigen to T cells at increased rates.⁸³ The potential role of rituximab-mediated B-cell depletion in patients with SLE has been addressed thus far in small, uncontrolled studies. Use of rituximab in doses smaller than those used in patients with lymphomas resulted in partial clinical response in a small group of patients.⁸⁴ Lack of response correlated best with the expression of low-affinity Fc γ RIIIa,⁸⁵ an observation that has significant implications in the design and potential patient tailoring of therapeutic mAbs. Rituximab appears to be useful also in the treatment of patients with SLE with glomerulonephritis.⁸⁶ The effect of B cell-depleting treatment on the levels of autoantibody is not clear from these small studies,⁸⁶⁻⁸⁸ and it is possible that the therapeutic effect of rituximab might depend on the ability of B cells to present autoantigen and activate T cells.⁸⁶

Rituximab treatment produced encouraging results in a small trial of patients with treatment-resistant anti-

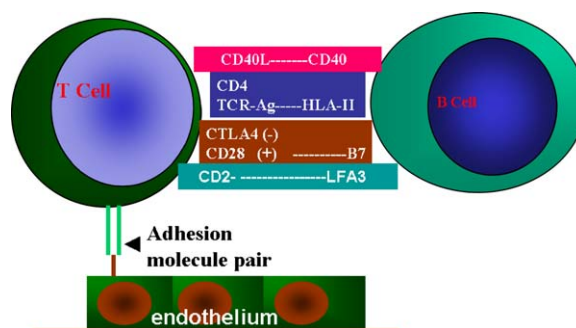


FIG 2. Disruption of cognate immune cell interaction. T and B cells interact through multiple pairs of costimulatory molecules. Humanized mAbs directed against these molecules might effectively disrupt the T-B cell interaction. Alternatively, decoy receptors can, in a similar fashion, limit the cognate interaction. Antibodies or decoy receptors can disrupt the homing, inappropriate in the case of inflammatory diseases, of lymphocytes to inflamed tissues.

proteinase 3-associated vasculitis. Rituximab treatment, combined with steroids or plasma exchange, produced B-cell depletion and significant decrease in the levels of circulating anti-proteinase 3 autoantibody. Clinical remission correlated with B-cell depletion in all patients. Reappearance of B cells in the peripheral blood correlated with relapses in some patients, but retreatment with rituximab caused B-cell depletion and induced a second clinical remission.⁸⁹

Disruption of costimulation

Abatacept (CTLA4Ig) in the treatment of patients with RA. T cells of the memory-activated phenotype are prominent in the rheumatoid synovium.⁹⁰ Molecules involved in T-cell costimulation, cytotoxic T lymphocyte-associated antigen 4 (CTLA4), CD80, and CD86, are expressed in increased amounts in inflamed RA joints.^{91,92} The principles that apply to blocking the CD80/CD86-costimulatory signal are shown in Fig 2.

CTLA4Ig and LEA29Y (a CTLA4Ig analog that differs from CTLA4Ig by only 2 amino acids but is 10 times more potent than CTLA4Ig in inhibiting costimulation) have been tested in clinical trials. Both constructs were reported to be safe, well tolerated, and effective in the treatment of RA, with LEA29Y producing slightly higher response rates.⁹³ Also, CTLA4Ig used in conjunction with methotrexate significantly enhanced the clinical response rate.⁹⁴

Anti-CD11a mAb (efalizumab) in the treatment of psoriasis. Efalizumab is a humanized anti-CD11a mAb that does not deplete T cells but blocks the interaction between the T-cell surface molecule lymphocyte function-associated antigen (LFA) 1 (composed of 2 subunits, CD11a and CD18) and intercellular adhesion molecule 1, which is found on the surface of antigen-presenting cells. Efalizumab tested in a placebo-controlled trial produced significant clinical improvement in patients with psoriasis, with spectacular improvement of the skin lesions.^{95,96}

LFA-3:Fc (alefacept) in the treatment of patients with psoriasis. Another pair of molecules with a significant role in T-cell activation-costimulation consists of CD2

(a pan-T-cell marker) and LFA-3, which is found on the surface of antigen-presenting cells. Alefacept is an FP composed of the LFA-3 molecule fused to the Fc portion of human IgG1. Alefacept blocks the delivery of CD2-initiated costimulatory signals to T cells and depletes memory-effector (CD45RO⁺) T cells.⁹⁷ A significant therapeutic efficacy for alefacept was recorded in patients with psoriasis in double-blind, placebo-controlled studies.^{98,99}

Anti-CD40L mAb in the treatment of patients with SLE. T cells, B cells, and monocytes from patients with SLE overexpress CD40L on their surface; CD40L overexpression plays a role in the production of autoantibodies *in vitro*.^{100,101} An anti-CD40L mAb infused in patients with SLE decreased the numbers of anti-dsDNA antibody-producing B cells, an effect that lasted for several months.¹⁰² The humanized anti-CD40L mAb IDEC-131 was tested in patients with SLE with mild to moderately severe SLE in a double-blind, placebo-controlled study, and although it was found to be safe, it failed to produce clinical improvement.¹⁰³ Another humanized anti-CD40L mAb, ruplizumab, reduced circulating anti-dsDNA antibody titers, increased the levels of C3, and decreased hematuria in patients with SLE nephritis, but the trial was discontinued because some patients experienced myocardial infarction.¹⁰⁴ Ruplizumab treatment resulted in elimination of peripheral blood CD38^{bright} antibody-secreting plasma cells in patients with SLE, and this effect correlated with decreases in serum anti-dsDNA antibody titers.¹⁰⁵

Anti-IgE mAb in the treatment of asthma. Omalizumab is a recombinant humanized mAb against IgE that recognizes the binding site of IgE for its high-affinity Fc receptor. Omalizumab therefore inhibits the attachment of free serum IgE onto mast cells and other effector cells. Eight randomized controlled trials that had registered 2037 patients with mild-to-severe allergic asthma (children and adults) with high serum IgE levels were recently meta-analyzed. Treatment with omalizumab significantly reduced serum IgE levels compared with placebo. Omalizumab administration led to significant reduction in inhaled steroid consumption compared with placebo and to significant increases in the numbers of patients able to reduce steroid dosages by greater than 50%, as well as in those who were able to totally discontinue daily steroids. Patients receiving omalizumab and steroids experienced fewer disease exacerbations than those treated with steroids alone. Omalizumab treatment was safe and well tolerated by children and adults.¹⁰⁶

CONSIDERATIONS AND FUTURE DIRECTIONS

The new biologic agents have induced salutary clinical effects and extended the therapeutic options of patients not responding to existing treatments. B-cell depletion treatment of patients with lymphomas has resulted in significant clinical responses and prolonged disease-free

survival. The use of anti-TNF- α antibodies and TNF decoy receptors in the treatment of patients with RA has improved significantly their quality of life. The future looks brighter than ever as the recorded success fuels efforts to optimize the use of the biologic agents and extend their use in other diseases.

Antibodies exert their effect either by (1) blocking cell-cell interactions, (2) blocking lymphokine-cell interaction, (3) stimulating action of cell membrane-bound molecules, or (4) destroying the target cell by activating complement or mediating antibody-dependent cell-mediated cytotoxicity (ADCC). In some cases more than one mode of action has been claimed. Because of the fact that complement activation and ADCC involve the Fc portion of IgG, it is obvious that IgGs can be modified genetically to improve complement activation and therefore provide more effective target cell killing or better binding to Fc receptors present on the surface of immune cells involved in ADCC. Alternatively, genetic screening of patients to identify those with high Fc receptor binding for the candidate therapeutic antibodies should eliminate unnecessary treatment of those who do not have high-affinity Fc receptors and therefore have low chances of responding. If the therapeutic mAb depends on complement activation to carry out its effect, then caution should be exercised in treating patients with SLE, who frequently have hypocomplementemia. It is possible that these patients will benefit from pretreatment aimed at correcting complement levels before the infusion of the therapeutic biologic agent.

Anti-TNF- α mAb treatment has been reported to increase the reactivation of tuberculosis because it might interfere with the cellular response against mycobacteria.¹⁰⁷ In addition to taking antituberculosis precautions to limit the exposure of high-risk patients, additional steps are needed to bypass this serious side effect. This is particularly relevant if anti-TNF- α treatment is to be introduced in populations with high rates of tuberculosis.

The effect of the B cell-depleting rituximab on RA and SLE, although impressive, harbors a number of problems in treating patients with relapses. Repeated elimination of B cells might lead to depletion of plasma cells and the development of hypogammaglobulinemia.¹⁰⁸

Progress in the treatment of human SLE has been slow, with no apparent biologic agent ready for FDA approval. Progress has been hampered by a number of factors, including clinical heterogeneity. Stratification of treated patients according to the major clinical manifestation might reveal a significant therapeutic effect that might not be obvious when all patients are lumped together. Clinical outcomes are difficult to measure because reliable biomarkers and surrogate markers are not available.¹⁰⁹

REFERENCES

1. Ortho Multicenter Transplant Study Group. A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. *N Engl J Med* 1985;313:337-42.
2. Norman DJ, Vincenti F, de Mattos AM, Barry JM, Levi DJ, Wedel NI, et al. Phase I trial of HuM291, a humanized anti-CD3 antibody, in patients receiving renal allografts from living donors. *Transplantation* 2000;70:1707-12.

3. Nashan B, Moore R, Amlot P, Schmidt AG, Abeywickrama K, Souillou JP. Randomized trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients [published erratum appears in *Lancet* 1997;350:1484]. *Lancet* 1997;350:1193-8.
4. Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. *N Engl J Med* 1998;338:161-5.
5. Kahan BD, Rajagopalan PR, Hall M. Reduction of the occurrence of acute cellular rejection among renal allograft recipients treated with basiliximab, a chimeric anti-interleukin-2-receptor monoclonal antibody. *Transplantation* 1999;67:276-84.
6. Nashan B, Light S, Hardie IR, Lin A, Johnson JR. Reduction of acute renal allograft rejection with daclizumab. *Transplantation* 1999;67:110-5.
7. Nussenblatt RB, Peterson JS, Foster CS, Rao NA, See RF, Letko E, et al. Initial evaluation of subcutaneous daclizumab treatments for noninfectious uveitis: a multicenter noncomparative interventional case series. *Ophthalmology* 2005;112:764-70.
8. Fisher JC Jr, Agosti JM, Opal SM, Lowry SF, Balk RA, Sadoff JC, et al. Treatment of septic shock with the tumor necrosis factor receptor: Fc fusion protein. The soluble TNF Receptor Study Group. *N Engl J Med* 1996;334:1697-702.
9. Impact-RSV Study Group. Pavilizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. *Pediatrics* 1998;102:531-7.
10. Mass R. The role of HER-2 expression in predicting response to therapy in breast cancer. *Semin Oncol* 2000;27:46-52.
11. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, et al. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. *J Clin Oncol* 1996;14:737-44.
12. Cobleigh M, Vogel C, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin Oncol* 1999;17:2639-48.
13. Slamon D, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783-92.
14. Vogel C, Cobleigh M, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, et al. First-line herceptin monotherapy in metastatic breast cancer. *Oncology* 2001;61:37-42.
15. Burnstein H, Kuter I, Campos S, Gelman RS, Tribou L, Parker LM, et al. Clinical activity of trastuzumab and vinorelbine in women with HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2001;19:2722-30.
16. Seidman A, Hudis C, Pierri M, Shak S, Paton V, Ashby M, et al. Cardiac dysfunction in the trastuzumab clinical trial experience. *J Clin Oncol* 2002;20:1215-21.
17. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;351:337-45.
18. Sridhar SS, Seymour L, Shepherd FA. Inhibitors of epidermal-growth-factor receptors: a review of clinical research with a focus on non-small-cell lung cancer. *Lancet Oncol* 2003;4:397-406.
19. Robert F, Ezekiel M, Spencer SA, Meredith RF, Bonner JA, Khazaeli MB, et al. Phase I study of anti-epidermal growth-factor receptor cetuximab in combination with radiation therapy in patients with advanced head and neck cancer. *J Clin Oncol* 2001;19:3234-43.
20. Xiong HQ, Rosenberg A, LoBuglio A, Schmidt W, Wolff RA, Deutsch J, et al. Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II Trial. *J Clin Oncol* 2004;22:2610-6.
21. Susman E. Rash correlates with tumour response after cetuximab. *Lancet Oncol* 2004;5:647.
22. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335-42.
23. Yang J, Haworth L, Sherry R. A randomized trial of bevacizumab, an anti-vascular-endothelial growth-factor antibody for metastatic renal cancer. *N Engl J Med* 2003;349:427-34.
24. Demidem A, Lam T, Alas S, Hariharan K, Hanna N, Bonavida B, et al. Chimeric anti-CD20 (IDEC-C2B8) monoclonal antibody sensitizes a B cell lymphoma cell line to cell killing by cytotoxic drugs. *Cancer Biother Radiopharm* 1997;12:177-86.
25. McLaughlin P, Grillo-Lopez J, Link BK, Czuczman MS, Williams ME, Heyman MR, et al. IDEC-C2B8 rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 1998;16:2825-33.
26. Piro LD, White CA, Grillo-Lopez AJ, Janakiraman N, Saven A, Beck TM, et al. Extended rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma. *Ann Oncol* 1999;10:655-61.
27. Colombat P, Salles G, Brousse N, Eftekhari P, Soubeyran P, Delwail V, et al. Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation. *Blood* 2001;97:101-6.
28. Hainsworth JD, Litchy S, Burris HA 3rd, Scullin DC Jr, Corso SW, Yardley DA, et al. Rituximab as first-line and maintenance therapy for patients with indolent non-Hodgkin's lymphoma. *J Clin Oncol* 2002;20:4261-7.
29. Czuczman MS, Grillo-Lopez AJ, McLaughlin P, White CA, Saleh M, Gordon L, et al. Clearing of cells bearing the bcl-2 [(t(14;18))] translocation from blood and marrow of patients treated with rituximab alone or in combination with CHOP chemotherapy. *Ann Oncol* 2001;12:109-14.
30. Coiffier B, Haioun C, Ketterer N, Engert A, Tilly H, Ma D, et al. Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicentre phase II study. *Blood* 1998;92:1927-32.
31. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002;346:235-42.
32. Ekstrand BC, Lucas JB, Horwitz SM, Fan Z, Breslin S, Hoppe RT, et al. Rituximab in lymphocyte-predominant Hodgkin disease: results of a phase II trial. *Blood* 2003;101:4285-9.
33. Dimopoulos MA, Zervas C, Zomas A, Kiamouris C, Viniou NA, Grigoraki V, et al. Treatment of Waldenström's macroglobulinemia. *J Clin Oncol* 2001;20:2326-33.
34. Berney T, Delis S, Kato T, Nishida S, Mittal NK, Madariaga J, et al. Successful treatment of post-transplantation lymphoproliferative disease with prolonged rituximab treatment in intestinal-transplant recipients. *Transplantation* 2002;74:1000-6.
35. Treon SP, Anderson KC. The use of rituximab in the treatment of malignant and non-malignant plasma cell disorders. *Semin Oncol* 2000;27:79-85.
36. Spina M, Jaeger U, Sparano JA, Talamini R, Simonelli C, Michieli M, et al. Rituximab plus infusional cyclophosphamide, doxorubicin, and etoposide in HIV-associated non-Hodgkin's lymphoma: pooled results from 3 phase 2 trials. *Blood* 2005;105:1891-7.
37. Ahrens N, Kingreen D, Seltsam A, Salama A. Treatment of refractory autoimmune haemolytic anemia with anti-CD20 (rituximab). *Br J Haematol* 2001;114:244-5.
38. Cooper N, Stasi R, Cunningham-Rundles S, Feuerstein MA, Leonard JP, Amadori S, et al. The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. *Br J Haematol* 2004;125:232-9.
39. Zecca M, DeStefano P, Nobili B, Locatelli F. Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated pure red cell aplasia and hemolytic anemia. *Blood* 2001;97:3995-7.
40. Gordon LI, Molina A, Witzig TE, Emmanouilides C, Raubitschek A, Dariff M, et al. Durable responses after ibritumomab tiutexan radioimmunotherapy for CD20+ B-cell lymphoma: long term follow-up of a phase I/II study. *Blood* 2004;103:4429-31.
41. Witzig TE, Flinn IW, Gordon LI, Emmanouilides C, Czuczman MS, Saleh MN, et al. Treatment with ibritumomab tiutexan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. *J Clin Oncol* 2002;20:3262-9.

42. Horning SJ, Younes A, Jain V, Kroll S, Lucas J, Podoloff D, et al. Efficacy and safety of tositumomab and iodine 131-tositumomab (Bexxar) in B-cell lymphoma progressive after rituximab. *J Clin Oncol* 2005;23:712-9.
43. Kaminski MS, Tuck M, Estes J, Kolstad A, Ross CW, Zasadny K, et al. 131I-tositumomab therapy as initial treatment for follicular lymphomas. *N Engl J Med* 2005;352:411-9.
44. Keating M, Flinn I, Jain V, Binet JL, Hillmen P, Byrd J, et al. Therapeutic role of alemtuzumab (campath-1H) in patients who have failed fludarabine: results of a large international study. *Blood* 2002;99:3554-61.
45. Lundin J, Kimby E, Björkholm M, Broliden PA, Celsing F, Hjalmar V, et al. Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia. *Blood* 2002;100:768-73.
46. Keating M, Cazin B, Coutre S, Birhary R, Kovacovics T, Langer W, et al. Campath-1H treatment of T-cell prolymphocytic leukemia in patients for whom at least one prior chemotherapy regimen had failed. *J Clin Oncol* 2002;20:205-13.
47. Perez-Simon JA, Kottaridis PD, Martino R, Craddock C, Caballero D, Chopra R, et al. Non-myeloablative transplantation with or without alemtuzumab: comparison between two prospective studies in patients with lymphoproliferative disorders. *Blood* 2002;100:3121-7.
48. Leonard JP, Coleman M, Ketas JC, Chadburn A, Ely S, Furman RR, et al. Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. *J Clin Oncol* 2003;21:3051-9.
49. Younes A, Hariharan K, Allen RS, Leigh BR. Initial trials of anti-CD80 monoclonal antibody (Galiximab) therapy for patients with relapsed or refractory follicular lymphoma. *Clin Lymphoma* 2003;3:257-9.
50. Borchmann P, Treml JF, Hansen H, Gottstein C, Schnell R, Staak O, et al. The human anti-CD30 antibody 5F11 shows in vitro and in vivo activity against malignant lymphoma. *Blood* 2003;102:3737-42.
51. Schnell R, Staak O, Borchmann P, Schwartz C, Matthey B, Hansen H, et al. A phase I study with an anti-CD30 ricin A-chain immunotoxin (Ki-4.dgA) in patients with refractory CD30+ Hodgkin's and non-Hodgkin's lymphoma. *Clin Cancer Res* 2002;8:1779-86.
52. Papadakis KA, Shaye OA, Vasiliauskas EA, Ippoliti A, Dubinsky MC, Birt J, et al. Safety and efficacy of adalimumab (D2E7) in Crohn's disease patients with an attenuated response to infliximab. *Am J Gastroenterol* 2005;100:75-9.
53. Chu CQ, Field M, Feldmann M, Maini RN. Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. *Arthritis Rheum* 1991;34:1125-32.
54. Neidel J, Schulze M, Lindschau J. Association between degree of bone-erosion and synovial fluid levels of tumor necrosis factor alpha in the knee-joints of patients with rheumatoid arthritis. *Inflamm Res* 1995;44:217-21.
55. Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, et al. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J* 1991;10:4025-31.
56. Targan SR, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. *N Engl J Med* 1997;337:1029-35.
57. Present DH, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezaand RA, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999;340:1398-405.
58. Sands BE, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004;350:876-85.
59. Rutgeerts P, Van Assche G, Vermeire S. Optimizing anti-TNF treatment in inflammatory bowel disease. *Gastroenterology* 2004;126:1593-610.
60. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, et al. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. *Lancet* 1999;354:1932-9.
61. Lipsky PE, van der Heijde DMF, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. *N Engl J Med* 2000;343:1594-602.
62. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Furst D, Weisman MH, et al. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. *Arthritis Rheum* 2004;50:1051-65.
63. Moreland LW, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, et al. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. *Ann Intern Med* 1999;130:478-86.
64. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. *N Engl J Med* 2000;343:1586-93.
65. Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, et al. Etanercept versus methotrexate in patients with early rheumatoid arthritis: two-year radiographic and clinical outcomes. *Arthritis Rheum* 2002;46:1443-50.
66. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* 1999;340:253-9.
67. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, et al. Adalimumab, a fully human anti-tumor necrosis factor α monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum* 2003;48:35-45.
68. Keystone EC, Kavanaugh AF, Sharp JT, Tannenbaum H, Hua Y, Teoh LS, et al. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy. *Arthritis Rheum* 2004;50:1400-11.
69. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor α -neutralizing agent. *N Engl J Med* 2001;345:1098-104.
70. Wolfe F, Michaud K. Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. *Arthritis Rheum* 2004;50:1740-51.
71. Braun J, Bollow M, Neure L, Seipelt E, Seyrekbasan F, Herbst H, et al. Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. *Arthritis Rheum* 1995;38:499-505.
72. Braun J, Brandt J, Listing J, Zink A, Alten R, Golder W, et al. Treatment of active ankylosing spondylitis with infliximab—a double-blind placebo controlled multicenter trial. *Lancet* 2002;359:1187-93.
73. Gottlieb AB, Li S, Evans R, Menter A. Infliximab in the treatment of psoriasis: results from the first 10 weeks of a phase II trial. Poster presented at: American Academy of Dermatology 61st Annual Meeting; San Francisco, CA: March 21-26, 2003.
74. Antoni C, Kavanaugh A, Kirkham B. The infliximab multinational psoriatic arthritis controlled study (IMPACT). *Arthritis Rheum* 2002;46(suppl):S381.
75. Antoni C, Kavanaugh A, Kirkham B. The infliximab multinational psoriatic arthritis controlled study (IMPACT). Substantial efficacy on synovitis and psoriatic lesions with or without concomitant DMARD therapy. *Ann Rheum Dis* 2003;62(suppl 1):90.
76. Mease PJ, Goffe BS, Metz J, VanderStoep A, Finck B, Burge DJ. Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomized trial. *Lancet* 2000;356:385-90.
77. Mease PJ, Kivitz AJ, Burch FX, Siegel EL, Cohen SB, Ory P, et al. Etanercept treatment of psoriatic arthritis: safety, efficacy, and effect on disease progression. *Arthritis Rheum* 2004;50:2264-72.
78. Leonardi CL, Powers JL, Matheson RT, Goffe BS, Zitnik R, Wang A, et al. Etanercept as monotherapy in patients with psoriasis. *N Engl J Med* 2003;349:2014-22.
79. Guerne PA, Zuraw BL, Vaughan JH, Carson DA, Lotz M. Synovium as a source of interleukin 6 in vitro: contribution to local and systemic manifestations of arthritis. *J Clin Invest* 1989;83:582-92.
80. Nishimoto N, Yoshizaki K, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: a multicenter, double-blind, placebo-controlled trial. *Arthritis Rheum* 2004;50:1761-9.

81. Mannon PJ, Fuss JJ, Mayer L, Elson CO, Sandborn WJ, Present D, et al. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* 2004;351:2069-79.
82. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N Engl J Med* 2004;350:2572-81.
83. Tsokos GC, Lioussis SNC. Immune cell signalling in lupus: activation, anergy and death. *Immunol Today* 1999;20:119-24.
84. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, et al. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. *Arthritis Rheum* 2004;50:2580-9.
85. Anolik JH, Campbell D, Felgar RE, Young F, Sanz I, Rosenblatt J, et al. The relationship of FcγRIIIa genotype to degree of depletion by rituximab in the treatment of systemic lupus erythematosus. *Arthritis Rheum* 2003;48:455-9.
86. Sfrikakis PP, Boletis JN, Lionaki S, Vigklis V, Fragiadaki KG, Iniotaki A, et al. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand. *Arthritis Rheum* 2005;52:501-13.
87. Anolik JH, Barnard J, Cappione A, Pugh-Bernard AE, Felgar RE, Looney RJ, et al. Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. *Arthritis Rheum* 2004;50:3580-90.
88. Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR, Isenberg DA. An open study of B lymphocyte depletion in systemic lupus erythematosus. *Arthritis Rheum* 2002;46:2673-7.
89. Keogh KA, Wylam ME, Stone JH, Specks U. Induction of remission by B lymphocyte depletion in eleven patients with refractory antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 2005;52:262-8.
90. Panayi GS. T-cell dependent pathways in rheumatoid arthritis. *Curr Opin Rheumatol* 1997;9:236-40.
91. Liu MF, Koshaka H, Sakurai H, Azuma M, Okumura K, Saito I. The presence of costimulatory molecules CD86 and CD28 in rheumatoid arthritis synovium. *Arthritis Rheum* 1996;39:110-4.
92. Liu MF, Yang CY, Li JS, Lai KA, Chao SC, Lei HY. Increased expression of down-regulatory CTLA-4 molecule on T lymphocytes from rheumatoid synovial compartment. *Scand J Immunol* 1999;50:68-72.
93. Moreland LW, Alten R, Van den Bosch F, Appelboom T, Leon M, Emery P, et al. Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA4-Ig and LEA29Y eighty-five days after the first infusion. *Arthritis Rheum* 2002;46:1470-9.
94. Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, et al. Treatment of rheumatoid arthritis by selective inhibition of T cell activation with fusion protein CTLA4Ig. *N Engl J Med* 2003;349:1907-15.
95. Leonardi CL, Papp KA, Gordon KB, Menter A, Feldman SR, Caro I, et al. Extended efalizumab therapy improves chronic plaque psoriasis: results from a randomized phase III trial. *J Am Acad Dermatol* 2005;52:425-33.
96. Gordon KB, Papp KA, Hamilton TK, Walicke PA, Dummer W, Li N, Bresnahan BW, et al. Efalizumab for patients with moderate to severe plaque psoriasis: a randomized controlled trial. *JAMA* 2003;290:3073-80.
97. Chamian F, Lowes MA, Lin SL, Lee E, Kikuchi T, Gilleaudeau P, et al. Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. *Proc Natl Acad Sci U S A* 2005;102:2075-80.
98. Krueger GC, Papp KA, Stough DB, Loven KH, Gulliver WP, Ellis CN. A randomized, double-blind, placebo-controlled, phase III study evaluating efficacy and tolerability of two courses of alefacept in patients with chronic plaque psoriasis. *J Am Acad Dermatol* 2002;47:821-33.
99. Lebwohl M, Christophers E, Langley R, Ortonne JP, Roberts J, Griffiths CEM. An international randomized, double-blind, placebo-controlled phase 3 trial of intramuscular alefacept in patients with chronic plaque psoriasis. *Arch Dermatol* 2003;139:719-27.
100. Desai-Mehta A, Lu L, Ramsey-Goldman R, Datta SK. Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J Clin Invest* 1996;97:2063-73.
101. Katsiari CG, Lioussis SNC, Souliotis VL, Dimopoulos MA, Manoussakis MN, Sfrikakis PP. Aberrant expression of the costimulatory molecule CD40 ligand on monocytes from patients with systemic lupus erythematosus. *Clin Immunol* 2002;103:54-62.
102. Huang W, Sinha J, Newman J, Reddy B, Budhai L, Furie R, et al. The effect of anti-CD40 ligand antibody on B cells in human systemic lupus erythematosus. *Arthritis Rheum* 2002;46:1554-62.
103. Kalunian KC, Davis JC Jr, Merrill JT, Totoritis MC, Wofsy D. Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2002;46:3251-8.
104. Boumpas DT, Furie R, Manzi S, Illei GG, Wallace DJ, Balow JE, et al. A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. *Arthritis Rheum* 2003;48:719-27.
105. Grammer AC, Slota R, Fischer R, Gur H, Girschick H, Yarbboro C, et al. Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154-CD40 interactions. *J Clin Invest* 2003;112:1506-20.
106. Walker S, Monteil M, Phelan K, Lasserson TJ, Walters EH. Anti-IgE for chronic asthma in adults and children. *Cochrane Database Syst Rev* 2004;CD003559.
107. Dinarello CA. Differences between anti-tumor necrosis factor-α monoclonal antibodies and soluble TNF receptors in host defense impairment. *J Rheumatol Suppl* 2005;74:40-7.
108. Tsokos GC. B cells—be gone. B-cell depletion in the treatment of rheumatoid arthritis. *N Engl J Med* 2004;350:2546-8.
109. Tsokos GC. Exploring complement activation to develop biomarkers for systemic lupus erythematosus. *Arthritis Rheum* 2004;50:3404-7.