Biologic Therapy: Hematopoietic Growth Factors, Retinoids, and Monoclonal Antibodies

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Biologic therapies are an increasingly important part of cancer treatment. In this chapter, we review the current status of studies of colony-stimulating factors (CSFs), erythropoietin (Epogen, Procrit), thrombopoietin, the retinoids, and monoclonal antibodies (MoAbs). The interferons, interleukin-2 (IL-2, aldesleukin [Proleukin]), and adoptive cellular immunotherapy are discussed in a separate chapter.

Hematopoietic Growth Factors
Retinoids
Monoclonal Antibodies
References

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Further work in this field will include study of the normal physiologic functions of cytokines. Understanding how dysregulation of cytokine function participates in the development of pathologic states may lead to the identification of additional clinical applications. Combination therapies exploiting complementary actions and interactions among naturally occurring molecules promise an added dimension to biologic therapy. Testing of new molecules, particularly those belonging to the ever-enlarging interleukin family, will continue and may yield additional therapeutic opportunities.

Hematopoietic Growth Factors

Hematopoietic growth factors are a family of glycoproteins with important regulatory functions in the processes of proliferation, differentiation, and functional activation of hematopoietic progenitors and mature blood cells [1]. The concept of humoral control of hematopoiesis dates back to the work of Carnot and Deflandre in 1906, who demonstrated that erythropoiesis is stimulated by a humoral factor, much later called erythropoietin, present in the serum of anemic rabbits [2]. In the 1960s, Bradley and Metcalf [3] developed an in vitro bone marrow culture system and observed the formation of cell colonies from hematopoietic progenitors. This hallmark development ultimately facilitated the characterization of a variety of hematopoietic growth factors known as CSFs. The subsequent development of molecular techniques led to genetic cloning of these factors and a large supply of recombinant molecules.

As shown in Table 1, the hematopoietic growth factors include erythropoietin, the CSFs, various interleukins (IL-1 to IL-13), stem-cell factor (SCF) newly described thrombopoietin (c-Mpl ligand) [4], and flt-3/flk-2, a growth factor for early progenitor cells [5]. The CSFs include granulocyte CSF (G-CSF, filgrastim [Neupogen]), granulocyte-macrophage CSF (GM-CSF, sargramostim [Leukine]), multipotential CSF (multi-CSF, also known as IL-3), and monocyte macrophage CSF (M-CSF, also known as CSF-1). Substantial clinical data have accrued on the four CSFs and erythropoietin, and these molecules have already had a major impact on therapy for hematologic and oncologic disease.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Target cell or activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-1</td>
<td>Induces cell growth of T-cells</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>Multipotential activity</td>
</tr>
<tr>
<td>Interleukin-3 (multi-CSF)</td>
<td>Hematopoietic progenitor cell growth</td>
</tr>
</tbody>
</table>
Interleukin-4
B-cell growth factor

Interleukin-5
Eosinophil differentiation factor

Interleukin-6
B-/T-cell growth and differentiation; megakaryocytes

Interleukin-7
B-/T-cell precursor growth

Interleukin-8
Neutrophil chemotactic factor

Interleukin-9
T-cell growth factor; megakaryocytes and burst-forming units-erythrocytes

Interleukin-10
Cytokine synthesis inhibition in natural-killer (NK) and T-cells

Interleukin-11
B-cells, multipotent progenitors, and megakaryocyte growth

Interleukin-12
Stimulates natural killer and cytotoxic T-cells

Granulocyte colony-stimulating factor (G-CSF)
Growth and activation of neutrophils

Erythropoietin
Erythroid growth and differentiation

SCF (c-kit ligand)
Stem-cell factor

Thrombopoietin
Thrombopoiesis

**Biology**

The hematopoietic growth factors have pleiotropic effects on the proliferation, differentiation, and functional activation of blood cells. They interact at various levels of the hematopoietic differentiation cascade, from multipotent progenitors to mature cells [6].

Each growth factor is encoded by a specific gene. The biologic effects of the growth factors are mediated through specific receptors on the surfaces of target cells. The receptor molecules are also encoded by specific genes, some of which have been cloned. The major cellular sources of the growth factors are monocytes, macrophages, T lymphocytes, endothelial cells, and fibroblasts. They are present and produce growth factors at multiple sites in the body. Erythropoietin production appears to be more restricted, however, with the predominant sources in the adult being the peritubular cells of the kidneys and the Kupffer cells of the liver.

Most growth factors stimulate more than one lineage. This ability to stimulate multiple cell lineages may be related to shared elements in receptor subunits. The receptors for GM-CSF, IL-3, and IL-5 are composed of a ligand-specific alpha chain and a common beta chain. Growth factors can be classified according to the level at which they act in hematopoiesis. Late-acting lineage-specific factors act on maturing cells. Erythropoietin, IL-5, and monocyte-macrophage CSF are examples of such factors. G-CSF regulates the proliferation and maturation of neutrophil progenitors but also acts with other factors to support the proliferation of primitive, dormant progenitors. GM-CSF, IL-3, and IL-4 are examples of intermediate-acting lineage-nonspecific factors that support the proliferation of multipotential progenitors. IL-6, IL-11, IL-12, G-CSF, and SCF act synergistically with IL-3 to induce dormant primitive progenitors to enter the cell cycle [7,8].

Erythropoietin promotes the proliferation and differentiation of committed erythroid progenitors. It
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may also interact with other hematopoietins to stimulate megakaryocytes in vitro. In vivo, erythropoietin produces consistent and sustained increases in erythropoiesis and hematocrit. In addition to stimulating cell proliferation and differentiation, CSFs also affect cell survival and functional activation. GM-CSF sustains viability and potentiates the functions of neutrophils, eosinophils, and macrophages [9]. G-CSF also potentiates the function of mature neutrophils but, unlike GM-CSF, appears not to increase the neutrophil half-life [10]. M-CSF enhances monocyte production of other cytokines, such as interferon, tumor necrosis factor, and CSFs themselves [11]. Indeed, virtually every function of granulocytes and macrophages that has been studied is modulated to some degree by G-CSF, GM-CSF, or M-CSF. IL-3 may regulate the function of eosinophils and monocytes [12]. Erythropoietin does not appear to alter the function of erythrocytes.

Clinical Applications
The availability of large quantities of recombinant growth factors has facilitated the exploitation of their biologic properties in the treatment of disease. G-CSF, GM-CSF, and erythropoietin have all been approved for clinical use for specific indications. A list of these indications and some investigational uses are summarized in Table 2.

recombinant Hematopoietic Growth Factors

G-CSF
Approved indications

- To decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs that are associated with a significant incidence of severe neutropenia with associated febrile episodes.

Indications under investigation

- To accelerate myeloid recovery in patients with myelodysplastic syndrome, AIDS (acquired immunodeficiency syndrome), marrow graft failure, peripheral blood stem-cell transplantation, congenital agranulocytosis, or malignancies not otherwise specified.

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Abrogation of Myelosuppression After Chemotherapy: The concept of using growth factors to mitigate the myelosuppressive effects of chemotherapy has generated a great deal of excitement. The potential of CSFs to reduce the morbidity and mortality from chemotherapy and to allow dose escalation has been energetically explored. Important studies have now established that G-CSF is able to abrogate or accelerate recovery from chemotherapy-induced neutropenia. For example, in a study of G-CSF following MVAC (methotrexate, vincristine [Oncovin], doxorubicin [Adriamycin, Rubex], and cisplatin [Platinol]) chemotherapy for urothelial tumors, the administration of therapy on schedule was possible in all patients receiving G-CSF but in only 29% of patients not receiving G-CSF [13]. The occurrence of mucositis was also diminished in the treated group.

In a study of patients with small-cell lung cancer, the duration of neutropenia was shorter and the number of febrile neutropenic episodes was lower in the G-CSF-treated group than in the placebo group [14]. The treated patients also spent fewer days in the hospital and received fewer antibiotics. Morstyn et al [15] confirmed the attenuation of neutropenia in patients treated with high-dose melphalan, even when the G-CSF was given as late as 8 days after chemotherapy.

GM-CSF has also produced encouraging results in the setting of chemotherapy-induced neutropenia. In comparisons with historic controls and in comparison between sequential courses of chemotherapy with and without GM-CSF, shorter durations of neutropenia and higher nadir neutrophil counts have been consistently observed in the treatment cycles with GM-CSF [16,17]. Despite the biologic data indicating that GM-CSF acts on early progenitors, no consistent enhancement of platelet recovery has been noted.

Autologous Bone Marrow Transplantation: Growth factors have been investigated for their ability to lessen myelosuppression associated with high-dose chemotherapy and autologous bone marrow rescue. G-CSF and GM-CSF have produced similar results in this setting. Studies have shown that time to neutrophil recovery in patients with Hodgkin's disease and lymphoid malignancies was reduced by these growth factors when compared with historic controls [18,19]. The number of febrile days and days in the hospital as well as the incidence of infection were also reduced. Toxic effects to organs were decreased, presumably as a result of shorter neutropenic periods. The ability of GM-CSF to enhance hematologic reconstitution has been confirmed in prospective, randomized trials [20,21].

G-CSF treatment after allogeneic bone marrow transplantation (BMT) has been shown to reduce the duration of neutropenia. Patients who received G-CSF had fewer infections and required less antibiotic treatment and shorter hospitalization than patients who did not receive G-CSF [22,23]. Clinical trials of G-CSF use after BMT have also indicated quicker granulocyte recovery [24-26].

Myelodysplastic Syndromes: The myelodysplastic syndromes (MDSs) are acquired primary or treatment-related neoplastic clonal stem-cell disorders characterized by ineffective hematopoiesis, dysplasia, and an increased propensity for leukemic transformation [27]. The French-American-British (FAB) classification identifies five MDS subtypes: refractory anemia, refractory anemia with ring sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia. Chromosomal abnormalities are commonly associated with these disorders. Among the most frequent abnormality is the loss of the long arm of chromosome 5 (5q-), on which is located a cluster of growth factor-related genes, including those coding for GM-CSF, M-CSF, M-CSF receptor, IL-3, IL-4, IL-5, and platelet-derived growth factor receptor. The significance of this association is under investigation. Because most patients with MDS die of infections and bleeding complications, therapy directed at increasing the number of circulating granulocytes and platelets and increasing red-cell mass appears very attractive.

Several studies have demonstrated a predictable increase in granulocyte count with GM-CSF and G-CSF in patients with MDS [28-35]. It is obvious from these studies that G-CSF or GM-CSF therapy can reduce the number of infections in patients with MDS who are neutropenic. Randomized trials comparing GM-CSF with placebo and G-CSF with observation have been reported. Most of these patients had fewer than 15% to 20% blasts in their bone marrow. There was no improvement in survival with either G-CSF or GM-CSF, and in the G-CSF study, overall survival of G-CSF-treated patients was shorter. The effect of GM-CSF on the cytopenias has been transient, disappearing in most cases with treatment cessation.

Studies of IL-3 in patients with MDS have been conducted in both Europe and the United States...
Biologic Therapy: Hematopoietic Growth Factors, Retinoids, and Monoclonal Antibodies

Toxicity

Acute Myeloid Leukemia: Estey et al [39] found no difference in the rate of infection or complete remission in patients with acute myeloid leukemia (AML) treated with fludarabine (Fludara), cytarabine, and G-CSF compared to historic controls treated with fludarabine and cytarabine alone. GM-CSF has been administered before chemotherapy in an attempt to induce leukemic blasts to enter the cell cycle, thus rendering them more susceptible to the drugs' cytotoxic effects. Despite evidence that blast production and ara-CTP (the active triphosphate derivative of cytarabine) incorporation into DNA are increased, the survival rate of patients receiving this therapy was inferior to that of historic controls [40]. Ongoing prospective, randomized trials are investigating the effect of GM-CSF before and during induction and consolidation and during the first two cycles of maintenance chemotherapy for newly diagnosed AML [41]. In an interesting study, Giralt et al [42] were able to obtain durable remissions in patients with AML who had relapsed after allogeneic BMT with G-CSF treatments. Other Hematologic Disorders: Aplastic anemia is characterized by peripheral blood cytopenias and bone marrow hypoplasia, thus representing another potential target for CSF therapy. Vadhan-Raj et al [43] showed that leukocyte counts improved in all patients treated with GM-CSF. No significant effects were observed in other cell lineages. The increases in leukocyte counts, which were accompanied by enhanced granulocyte function, persisted only for the duration of the GM-CSF infusion. Other investigators demonstrated that GM-CSF therapy could not induce hematopoiesis in patients with the most severe form of aplastic anemia [44]. Very low doses of GM-CSF (5 to 20 µg/m²/d) have also successfully increased neutrophil counts in nearly 50% of patients with aplastic anemia [45]. Further investigation of GM-CSF in combination with other growth factors is warranted. IL-3 has also been studied as a therapy for aplastic anemia. Ganser and co-workers [46] found increases in leukocyte counts in all nine patients treated. Platelet responses varied and included a transient decline in two patients. Kurzrock et al [36,47] have reported neutrophil responses in approximately one third of patients and platelet or reticulocyte responses in 10% of patients with aplastic anemia receiving IL-3. These effects were often modest and delayed in onset, although they persisted longer after cessation of IL-3 than did the effects of GM-CSF therapy. IL-3 combined with GM-CSF appears promising for increasing platelet counts as well as granulocyte counts in patients with aplastic anemia [48].

G-CSF has revolutionized therapy for the chronic neutropenias. A number of reports on cyclic neutropenia, congenital neutropenia, and idiopathic neutropenia have confirmed neutrophil responses in virtually 100% of patients treated with G-CSF, and resolution of infectious complications has been observed [47]. GM-CSF produces less satisfactory results, with eosinophilia predominating over neutrophil responses; one report suggested, however, that very low doses (0.3 µg/kg/d) may be more effective than standard doses [49].

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Anemia: Erythropoietin was first used clinically in patients with anemia and chronic renal failure who were undergoing dialysis. It has shown consistent, sustained success in improving the hematocrit in this setting, presumably because the production of erythropoietin is impaired along with the impairment of other renal functions [50]. In contrast to the clinical settings in which CSFs are used, renal failure represents a true erythropoietin deficiency state. Anemia frequently accompanies malignant disease, although endogenous erythropoietin production is not usually impaired in this setting. Miller et al [51] have shown, however, that the magnitude of the erythropoietin response in patients with cancer and anemia is blunted, compared with that of patients with iron-deficiency anemia. Patients with hematologic malignancies and anemia can have a wide range of endogenous erythropoietin levels, and a variety of contributory mechanisms of anemia probably exist [47-52]. Nonetheless, treatment of this group with erythropoietin has produced some encouraging data. In one study, 11 of 13 anemic patients with multiple myeloma responded to erythropoietin, with improvements in hemoglobin levels [53]. Less promising results were obtained in patients with MDS. Indeed, in our studies, only 2 of 16 patients with MDS responded [52]. Erythropoietin has been successful, however, in abrogating the anemia associated with therapy for acquired immunodeficiency syndrome (AIDS) therapy [54].

Toxicity

The toxic effects of growth factors have been reviewed recently [20]. Unfortunately, no direct...
comparisons of G-CSF and GM-CSF have been performed. However, comparing their toxicities in different studies using equally effective doses in the same clinical settings reveals greater toxicity after GM-CSF administration [20]. The first dose of GM-CSF may be accompanied by flushing, tachycardia, hypotension, dyspnea, hypoxemia, musculoskeletal pain, and nausea in a small subset of patients. This first-dose effect is more common with intravenous administration. More important, GM-CSF can induce fever and chills, which are difficult to distinguish from the signs and symptoms of infection. Other complaints include lethargy, myalgia, bone pain, and anorexia, but they are usually mild. A capillary leak syndrome characterized by edema, effusions, and inflammation may develop. Other rare side effects of GM-CSF include exacerbation of thrombocytopenia and reactivation of autoimmune disorders. Doses lower than 5 µg/kg are usually reasonably well tolerated, whereas higher doses more frequently produce severe side effects.

The most frequent G-CSF toxicity is bone pain, which is more common with intravenous administration. Fever, rash, and arthralgia are uncommon. Mild splenomegaly may occur, particularly with long-term use of G-CSF. Thrombocytopenia also has been reported, albeit rarely. Doses between 1 and 20 µg/kg are usually well tolerated. At doses higher than 30 µg/kg, excessive leukocytosis typically occurs.

Interleukin-3 (IL-3) is well tolerated at doses lower than 1,000 µg/m²/d, although low-grade fever and chills occur in a high percentage of patients. Headaches are more frequent at higher doses. Uncommon side effects include bone pain, edema, and nausea, all of which are generally mild. Erythropoietin has caused very few side effects, even with long-term usage. Iron deficiency does occur, however, in patients with insufficient iron stores.

**Retinoids**

Retinoids are substances structurally or functionally related to vitamin A, or retinol. They exert profound effects on the growth, maturation, and differentiation of many cell types, both in vivo and in vitro [55]. Vitamin A is a vital factor in normal embryogenesis, and it influences limb development and growth pattern. The effects of retinoids are mediated by two classes of nuclear retinoic acid receptors, termed RAR and RXR. Each of these subclasses has subtypes designated alpha, beta, and gamma. These receptors are ligand-inducible, transcription-enhancer factors belonging to the nuclear receptor superfamily, which includes thyroid and steroid hormone receptors. Cytoplasmic retinoic acid-binding proteins are important in the mediation of some of the effects of retinoids. However, not all tissues responsive to retinoic acid possess this protein (e.g., HL-60 cells).

Retinoids reportedly induce differentiation and/or suppression of proliferation of many cell lines, including embryonal carcinoma, leukemia, melanoma, neuroblastoma, and breast carcinoma. The best studied line is HL-60, which can be induced to differentiate to granulocytes expressing such functional characteristics as phagocytosis, complement receptors, chemotaxis, and the ability to reduce nitroblue tetrazolium. Synergy has been seen when retinoids were combined with vitamin D and its analogs as well as in combination with other cytokines [56,57].

**Clinical Applications**

**Chemoprevention:** Sporn et al [58] were the first to use the term chemoprevention, which can be defined as the use of specific natural or synthetic chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer. This subject was recently reviewed by Lippman et al [59].

A significant amount of data has accumulated over the past several years regarding the role of retinoids as chemopreventive agents, mainly in the setting of head and neck and lung cancers. Patients with a history of cancer of the head and neck and lungs are at a significantly increased risk of developing a second primary tumor. This is thought to be due to the “field cancerization” effect, in which diffuse injury to the epithelia results from the carcinogen exposure.

The ineffectiveness of current therapy for lung cancer also prompted studies evaluating the role of retinoids in preventing lung cancer development [60]. In a randomized, placebo-controlled trial, Hong and colleagues [61] have shown a significant decline in the incidence of second primary tumors with the use of isotretinoin 50 to 100 mg/m²/d for 1 year in patients with treated head and neck cancers. Pastorino et al [62] evaluated retinyl palmitate (300,000 IU/d for 1 year) in patients with resected stage I lung cancer. There was a significant decline in the number of second primary tumors in patients who received retinyl palmitate, compared with a control group who received placebo. Bolla et al [63], however, could not demonstrate any beneficial effects of etretinate, a synthetic retinoid, on the incidence of second primary tumors in patients with a history of squamous-cell carcinoma of the oral cavity or oropharynx. The role of retinoids as chemopreventive...
agents will be better defined upon completion of the large multicenter trials now under way (the North American Intergroup Lung Study and the European EUROSCAN study) [64]. In addition to their use in chemoprevention, retinoids have been used to treat various malignant diseases. Table 3 summarizes some of the data from these studies.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Response rate (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute promyelocytic leukemia</td>
<td>80-100</td>
<td></td>
</tr>
<tr>
<td>Juvenile chronic myelogenous leukemia</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin's lymphoma</td>
<td>40-50</td>
<td>Small number of patients</td>
</tr>
<tr>
<td>Squamous-cell carcinoma of the cervix</td>
<td>50-60</td>
<td>Treatment combined with alpha interferon</td>
</tr>
<tr>
<td>Squamous-cell carcinoma of skin</td>
<td>60-70</td>
<td>Improved response in combination with alpha interferon</td>
</tr>
</tbody>
</table>

**Acute Promyelocytic Leukemia:** Classified as M3 in the FAB classification system, acute promyelocytic leukemia (APL) is a relatively uncommon leukemia. It is characterized by a propensity for coagulopathy and a high death rate from bleeding diatheses during induction. Morphologically, APL cells have abundant dense granules and Auer rods. Cytogenetic analysis reveals t(15;17) in the vast majority of patients. In instances in which no cytogenetic abnormality is present, more sensitive techniques, such as Southern blotting and reverse transcriptase-polymerase chain reaction amplification, reveal a characteristic RAR-alpha/PML gene rearrangement. The retinoic acid receptor RAR-alpha gene is located on the long arm of chromosome 17. The PML gene is located on chromosome 15. The t(15;17) translocation produces a recombinant gene, PML-RAR-alpha. The PML-RAR-alpha gene product is widely thought to be responsible for the differentiation block seen in APL blasts. The underlying mechanism or mechanisms of action of the block are still unknown [65]. APL has traditionally been treated with anthracyclines with or without cytarabine. This treatment has a high complete remission rate, with a reported long-term survival of 30% to 40% [66]. Most treatment failures are due to early deaths from bleeding and infectious complications secondary to the aplastic state produced by induction chemotherapy.

All-trans retinoic acid (ATRA) has been successfully used to achieve complete remission in most patients with APL morphology and PML-RAR-alpha gene rearrangement [67]. ATRA therapy results in the terminal differentiation of APL blasts into functionally mature granulocytes. Furthermore, within the first 48 hours of treatment, ATRA therapy corrects the coagulopathy associated with APL. ATRA induces complete remission in 3 to 4 weeks of therapy in more than 90% of patients with APL [68]. De novo resistance to ATRA is very rare. ATRA therapy, unlike chemotherapy, does not produce an aplastic phase, thereby reducing the occurrence of infectious complications. ATRA therapy alone, however, has a high early (1 to 12 months) relapse rate, approaching virtually 100%. Recent studies have suggested that a combination of ATRA and chemotherapy may be better than either alone in terms of survival [69,70]. Patients whose disease relapses after ATRA therapy are generally resistant to retreatment with ATRA.

The mechanisms underlying ATRA sensitivity of APL blasts and the subsequent development of resistance are still not well defined. The PML-RAR-alpha gene product has been implicated in both leukemogenesis and response to ATRA. The development of resistance to ATRA is at least partly explained by the decline in drug levels due to the increased metabolism of ATRA during prolonged therapy [65].

**Other Malignancies:** Using a combination of alpha interferon (IFN-alfa) and isotretinoin in patients with advanced squamous-cell carcinoma of the skin and cervix, Lippman et al [71,72] obtained significant responses. Cheng et al [73] investigated the use of 13-cis retinoic acid in patients with non-Hodgkin’s lymphoma. In this study, T-cell lymphomas responded, but B-cell lymphomas did not. Kurzrock et al [74] demonstrated no benefit of ATRA treatment for patients with MDS. Castleberry et
al [75] used ATRA to treat juvenile chronic myelogenous leukemia and obtained lasting remissions in 5 of 10 patients (two complete remissions and three partial remissions).

**Toxicity**

Retinoids are highly teratogenic compounds and therefore must be used with the utmost caution in women of child-bearing age. Apart from this serious side effect, retinoic acid toxicities are generally mild. Drying of the skin and mucous membranes occurs in virtually all patients. Arthralgias, hypertriglyceridemia, elevated liver function values, skin rashes, and mild hair loss occur in 10% to 25% of patients. More rarely, corneal opacities, exfoliation, pseudotumor cerebri, and proteinuria have occurred.

**Retinoic Acid Syndrome**

Retinoic acid syndrome (RAS), which is manifested as fever, respiratory distress, hyperleukocytosis, edema, pleural and pericardial effusions, hypotension, and renal failure, may occur in up to 20% of patients with APL treated with ATRA [76]. It occurs mainly within the first 3 weeks of treatment. Patients exhibiting hyperleukocytosis at the start of treatment have an increased risk of developing RAS. ATRA administered concomitantly with chemotherapy is beneficial in these patients [77]. High doses of corticosteroids given at the first sign of RAS were also beneficial.

**Monoclonal Antibodies**

Hybridoma technology has provided a reliable system for the production of large quantities of antibodies of defined specificity (MoAbs). Hybridoma formation requires the fusion of B lymphocytes from immunized mice and myeloma cells, resulting in immortalized MoAb-producing cells [78]. Human B lymphocytes have also been used to create chimeric hybridomas; however, this technique remains difficult. Genetically engineered chimeric “humanized” MoAbs (MoAbs with a murine complementarity-determining region attached to a human immunoglobulin molecule) have been developed in an attempt to reduce the immunogenicity of murine MoAbs.

Antibodies are capable of binding with high affinity to specific determinants. The consequences of antibody binding vary and include (1) direct neutralization of the target (as in the case of certain viruses or toxins), (2) indirect mediation of immune damage by means of complement activation, and (3) activation of cellular cytotoxicity by other immunocompetent cells (antibody-dependent cell cytotoxicity). Variables influencing the outcome include the antibody isotype, binding affinity, and target epitope frequency.

The first step in monoclonal antibody therapy is to choose an appropriate target for antibody specificity. The optimum target is a tumor-specific surface antigen not expressed on normal tissue. For example, some B-cell malignancies demonstrate unique antigenic determinants by virtue of the clonal expression of immunoglobulin molecules on the malignant cells. Unique tumor determinants such as these are not found in most tumors, however, so other tumor-associated antigens have been targeted. They include differentiation antigens that are normally expressed only at specific stages of differentiation and on limited cell lineages (eg, common acute lymphoblastic leukemia antigen CD10 [CALLA], anti-T-activated cell antibody [anti-TAC]). Oncofetal antigens, including carcinoembryonic antigen (CEA) and alpha-fetoprotein, are alternative targets.

Many MoAbs do not adequately effect immune destruction of target cells alone. Thus, MoAbs have been conjugated with other agents that are cytotoxic, including toxins, chemotherapeutic agents, and radioisotopes. The resultant immunotoxins or immunoconjugates may have the potential to improve targeted cell therapy significantly. Potent protein toxins, such as ricin and diphtheria, have also been used, because they are active only after internalization into the cell. Common radioisotopes include iodine 131, yttrium 90, indium 111, and rhenium 186.

Another strategy for MoAb treatment involves targeting growth-factor receptors. Such antireceptor MoAbs compete with natural ligands functioning as autocrine growth factors, thus impairing tumor growth. Examples include anti-IL-2 receptor and antiepidermal growth factor receptor MoAbs. This strategy may also be used to deliver conjugated molecules via the cell receptor system.

Finally, MoAbs are being developed for use as diagnostic tools to locate residual tumor after treatment and to uncover occult tumors not localized by conventional methods.

**Clinical Trials**

The status of MoAb therapy for cancer was reviewed recently [79-91]. Table 4 summarizes some of the studies performed with MoAbs in various malignancies [90-114]. As is obvious at a glance, most of these studies were pilot or phase-I studies with small numbers of patients. The results generally have been disappointing. However, these studies have demonstrated that MoAbs can be safely administered to humans with acceptable toxicity.
<table>
<thead>
<tr>
<th>Study</th>
<th>MoAb</th>
<th>Disease</th>
<th>Number of responders/number treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheinberg et al [90]</td>
<td>M195 (Cd33)</td>
<td>AML</td>
<td>0/10</td>
</tr>
<tr>
<td>Dyer et al [92]</td>
<td>CAMPATH</td>
<td>ALL</td>
<td>3/5</td>
</tr>
<tr>
<td>Carone et al [92]</td>
<td>Humanized M195</td>
<td>AML and blastic</td>
<td>—</td>
</tr>
<tr>
<td>Schwartz et al [94]</td>
<td>Iodine-131 M195</td>
<td>ALL</td>
<td>—</td>
</tr>
<tr>
<td>Vitetta et al [95]</td>
<td>B-43-Pokeweed anti-CD-19 viral protein</td>
<td>ALL</td>
<td>—</td>
</tr>
<tr>
<td>Foon et al [96]</td>
<td>T101 (CDS)</td>
<td>ALL</td>
<td>—</td>
</tr>
<tr>
<td>Klien et al [97]</td>
<td>Anti-IL-6</td>
<td>Multiple myeloma</td>
<td>0/1</td>
</tr>
<tr>
<td>Hu et al [98]</td>
<td>Lym-1</td>
<td>B-cell lymphoma</td>
<td>0/10</td>
</tr>
<tr>
<td>Brown et al [99]</td>
<td>Anti-idiotype</td>
<td>B-cell lymphoma</td>
<td>9/17</td>
</tr>
<tr>
<td>Miller et al [100]</td>
<td>Leu-1 (CD5)</td>
<td>T-cell lymphoma</td>
<td>5/7</td>
</tr>
<tr>
<td>Dillman et al [101]</td>
<td>T101 (CD5)</td>
<td>T-cell lymphoma</td>
<td>0/12</td>
</tr>
<tr>
<td>Press et al [103]</td>
<td>Iodine-131-labeled IF5, MB-1 + auto</td>
<td>B-cell lymphoma</td>
<td>17/19</td>
</tr>
<tr>
<td>Ryan et al [104]</td>
<td>Several</td>
<td>Breast</td>
<td>0/10</td>
</tr>
<tr>
<td>Halpern et al [105]</td>
<td>Anti-PAP</td>
<td>Prostate</td>
<td>0/19</td>
</tr>
<tr>
<td>Goodman et al [106]</td>
<td>Anti-L6</td>
<td>Ovary</td>
<td>0/9</td>
</tr>
<tr>
<td>Jacobs et al [107]</td>
<td>Rhenium-186 relabeled NR-LU10 (intraperitone)</td>
<td>Ovary</td>
<td>4/17</td>
</tr>
<tr>
<td>Dillman et al [108]</td>
<td>Anti-CEA</td>
<td>Colorectal</td>
<td>0/30</td>
</tr>
<tr>
<td>Sears et al [109]</td>
<td>17-1A</td>
<td>Colorectal</td>
<td>1/60</td>
</tr>
<tr>
<td>Mellstedt et al [110]</td>
<td>17-1A</td>
<td>Colorectal</td>
<td>1/52</td>
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<tr>
<td>Takahashi et al [111]</td>
<td>A7-NCS</td>
<td>Colorectal</td>
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<tr>
<td>Halpern et al [112]</td>
<td>Anti-p97</td>
<td>Lung, small-cell</td>
<td>1/19</td>
</tr>
<tr>
<td>Dillman et al [108]</td>
<td>Anti-p240</td>
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</tr>
<tr>
<td>Houghton et al [113]</td>
<td>R24 (GD3)</td>
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<td>4/21</td>
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<td>Lynch et al [114]</td>
<td>N901-bR</td>
<td>Melanoma</td>
<td>0/24</td>
</tr>
</tbody>
</table>

ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; BMT = bone marrow transplantation; CEA = carcinoembryonic antigen; PAP = prostatic acid phosphatase.
The initial failure of MoAb therapy may be attributable, at least in part, to the early development of human antimurine antibodies in recipients. These antibodies develop in 90% of patients after more than two treatments, greatly reducing the ability of the MoAbs to reach their target. Avascular tumor beds also prevent MoAbs from reaching the malignant tissue. Heterogeneity of tumor antigens and the mutation of antigens over time also abrogate the effectiveness of this target-specific therapy. Strategies to overcome these problems are being investigated and include the development of chimeric mouse/human MoAbs to reduce their immunogenicity.

Immunotoxins and immunoconjugates have met with modest success in vivo. Radionuclide conjugates have been used in the treatment of ovarian cancer, leukemia (anti-CD33 MoAb), non-Hodgkin's lymphoma, and brain tumors. Microscopic disease has proven more amenable to this therapy than has gross tumor. Immunotoxin therapy has most commonly used ricin. Encouraging results were observed when an anti-CD5 conjugate was administered to patients with chronic lymphocytic leukemia. Trials of ricin-linked MoAb therapy in patients with breast or colon cancer have been frustrated by the development of human antimurine antibodies and the consequent loss of MoAb efficacy. MoAbs have also been used after autologous BMT to purge tumor cells ex vivo in autologous bone marrow therapy or to select for progenitor cells in allogeneic BMT. In addition, MoAbs have been used to deplete the marrow of CD8+ cells to reduce the incidence of graft-vs-host disease. MoAbs remain a conceptually logical approach to therapy. Further understanding of tumor immunology and advances in technology will facilitate the ongoing development of these strategies. Presently, clinical trials using chimeric “humanized” MoAbs, immunotoxins, and MoAbs conjugated to radioisotopes for diagnosis and therapy of malignant diseases are underway.

References:


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