Perspectives in Cancer Research

Biological Response Modifiers: The New Immunotherapy

Kenneth A. Foon

Division of Clinical Immunology, Roswell Park Memorial Institute, and Department of Medicine, State University of New York at Buffalo, Buffalo, New York 14263

Immunotherapy is divided into two overlapping categories, active and passive. The goal of active immunotherapy is the stimulation of host antitumor immunity, either cellular or humoral. This can be accomplished in a direct or specific fashion by using tumor vaccines to generate an immune response to tumor-associated antigens. Nonspecific antitumor immunity can be propagated by compounds such as BCG. Passive immunotherapy relies on the administration of biologically active agents with innate antitumor properties, such as antibodies reactive with growth factor receptors. In most instances, host immunity is an important cofactor in active immunotherapy.

In addition, some agents, such as antibodies, can exert antitumor effects via active or passive mechanisms. The complex immune circuits that are set into motion by these therapies account for the imperfect, but nonetheless useful division into active and passive types.

Immunotherapy is very effective in certain animal model systems and it has been used to treat human cancers for several decades (1). In the 1960s and 1970s trials of nonspecific immunostimulants including BCG or specific allogeneic or autologous vaccines were evaluated with early promising reports (2-5). Unfortunately, none of these approaches has been shown to be effective in controlled trials (6, 7).

During the past decade, renewed interest in immunotherapy has been stimulated by genetic engineering and mass cell culture and improved techniques in protein and nucleic acid sequencing. These have made available highly purified molecules including interferons, interleukins, tumor necrosis factor, and hematopoietic growth factors. Also, hybridoma technology (8) has generated murine monoclonal antibodies directed against tumor-associated antigens. The term "biological response modifiers" (9) is often used to refer to these newer approaches to immunotherapy.

Interferons

Interferon was initially identified as a soluble factor able to inhibit infection of chick chorioallantoic membranes by influenza A virus (10). Subsequent studies have shown the interferons to be a family of closely related proteins and glycoproteins. In addition to their antiviral activity these molecules are potent regulators of cell gene expression, structure, and function. They also exhibit direct antiproliferative activity. These properties underlie the current interest in interferon as an anticancer agent.

Three major species of human interferon are identified: α, β, and γ (11) (Table 1). α-Interferon is produced by leukocytes (B-cells, T-cells, null cells, and macrophages) following exposure to B-cell mitogens, viruses, foreign cells, or tumor cells. β-Interferon is produced by fibroblasts following exposure to viruses or foreign nucleic acids. γ-Interferon is produced by T-lymphocytes following stimulation of T-cell mitogens, specific antigens, or IL-2 (13). Complete nucleotide sequences for the α-, β-, and γ-interferon genes are known, and amino acid sequences have been deduced (14-16). Sixteen distinct α-interferon genes are described; each encodes a protein of approximately 166 amino acids (14). Only a single β-interferon gene has been identified encoding a protein of 166 amino acids; similarly there appears to be a single γ-interferon gene encoding a protein of 144 amino acids (15-17).

The α-interferon first used in clinical trials was obtained from Sendai virus-stimulated buffy coat leukocytes. Its purity was about 1% (10⁶ units/mg protein; 1 unit of interferon is approximately the amount that reduces viral replication in cell culture by one-half) (18). Use of high performance liquid chromatography, two dimensional polyacrylamide gel electrophoresis, and immune affinity chromatography has resulted in the purification of α-interferon to homogeneity (10⁶ units/mg protein) (19, 20). Use of recombinant DNA technology can produce large quantities of pure α-interferon (21).

Large scale production of β- and γ-interferons is more recent and clinical trials are limited. α-Interferon has been extensively studied for the past decade in both basic science and clinical research and is among the most potent biological agents ever administered to humans. Although antitumor activity is detected in some solid malignancies in vitro and in vivo (22-24), the most impressive responses are in hematological malignancies (12).

The mechanisms of interferon-mediated antitumor effects in murine models and humans are unclear. Putative mechanisms include a direct antiproliferative effect on the tumor, induction or augmentation of a host effector mechanism, such as NK and monocyte cytotoxicity, and induction or augmentation of expression of membrane antigens on tumor cells which facilitate subsequent immune recognition by the host.

α-Interferon has moderate activity in a limited number of solid tumors (Table 2). It is active in bladder cancer when instilled directly at very high doses (57-59). It is also active in acquired immunodeficiency syndrome-related Kaposi sarcoma (45-47), renal cell carcinoma (40-44), malignant melanoma (27-32), and carcinoid tumors (90). There is little evidence of activity in the common cancers such as breast and colon cancer and only limited activity for squamous cell tumors of the head and neck and lung (33-39, 48-52, 48, 53, 54, 60, 61). Clearly, α-interferon as a single agent will not have a major impact in the therapy of solid tumors. Current directions of interferon research include combinations with other biologicals and in some cases cytotoxic drugs.

α-Interferon has had its greatest impact in the treatment of certain hematological malignancies. Approximately 90% of patients with hairy cell leukemia respond to α-interferon (64-71) with a normalization of blood counts. Improvement in natural killer cell activity and immunological surface marker...
Solid malignancies
Hematological malignancies
Hairy cell leukemia

Expression parallels immune recovery (68). Complete responses are rare with α-interferon and there are no cures. However, patients have durable responses. Side effects are few because only low doses of interferon are required for response. α-Interferon can be discontinued in most patients after a response is achieved. Most patients relapse over a period of 6 months to 2 years (71) but respond to additional treatment with interferon. Pentostatin is also very effective therapy in hairy cell leukemia and is an alternative in patients who fail to respond or become resistant to α-interferon (72). Studies to assess the standard

Table 1 Interferons in clinical use

<table>
<thead>
<tr>
<th>Type</th>
<th>Subtype (new nomenclature)</th>
<th>Source</th>
<th>Purity (%)</th>
<th>Amino acid differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>Leukocyte (IFN-α[LE])&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Leukocytes from normal blood</td>
<td>&lt;1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Arginine at position 23; deletion at position 44 when compared to other α subtypes.</td>
</tr>
<tr>
<td></td>
<td>Lymphoblastoid (IFN-α[α]-N1), Woberton&lt;sup&gt;a&lt;/sup&gt; (Broughton Welcombe Co.)</td>
<td>Lymphoblastoid cells in culture</td>
<td>&lt;1&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recombinant α2 (IFN-α[α]-2b), Intron A&lt;sup&gt;a&lt;/sup&gt; (Schering Corp.)</td>
<td>Transformed Escherichia coli</td>
<td>&gt;95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recombinant αA (IFN-α[α]-2a), Roferon-Α&lt;sup&gt;a&lt;/sup&gt; (Hoffmann-La Roche, Inc.)</td>
<td>Transformed E. coli</td>
<td>&gt;95</td>
<td>Lysoleucine at position 23; deletion at position 44.</td>
</tr>
<tr>
<td></td>
<td>Recombinant αD (IFN-α[D])</td>
<td>Transformed E. coli</td>
<td>&gt;95</td>
<td>29 variations from αA</td>
</tr>
<tr>
<td></td>
<td>Recombinant α2arg (IFN-α[α]-2c)</td>
<td>Transformed E. coli</td>
<td>&gt;95</td>
<td>Arginine at position 23.</td>
</tr>
</tbody>
</table>

β

<table>
<thead>
<tr>
<th>Source</th>
<th>Purity (%)</th>
<th>Amino acid differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast (IFN-β)</td>
<td>Fetal foreskin fibroblast in culture</td>
<td>&lt;1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Recombinant 3β (IFN-β&lt;sub&gt;3β&lt;/sub&gt;)</td>
<td>Transformed E. coli</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Recombinant 3β (IFN-β&lt;sub&gt;3β&lt;/sub&gt;)</td>
<td>Transformed E. coli</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

γ

<table>
<thead>
<tr>
<th>Source</th>
<th>Purity (%)</th>
<th>Amino acid differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune (IFN-γ)</td>
<td>T-lymphocytes from normal blood</td>
<td>&lt;1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Recombinant γ (IFN-γ)</td>
<td>Transformed E. coli</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

Table 2 Clinical trials with α-interferon

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. of evaluable patients</th>
<th>CR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PR</th>
<th>MR</th>
<th>% of total response</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid malignancies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteogenic sarcoma</td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>25, 26</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>185</td>
<td>7</td>
<td>14</td>
<td>2</td>
<td>11</td>
<td>27-32</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>187</td>
<td>0</td>
<td>14</td>
<td>10</td>
<td>7</td>
<td>33-39</td>
</tr>
<tr>
<td>Renal cell</td>
<td>252</td>
<td>37</td>
<td>28</td>
<td>17</td>
<td>40-44</td>
<td></td>
</tr>
<tr>
<td>Kaposi’s sarcoma (AIDS-related)</td>
<td>120</td>
<td>14</td>
<td>22</td>
<td>36</td>
<td>45-47</td>
<td></td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>65</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>48-52</td>
<td></td>
</tr>
<tr>
<td>Carcinoid</td>
<td>9</td>
<td>0</td>
<td>6</td>
<td>67</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small cell</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Non-small cell</td>
<td>70</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>48, 54</td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>42</td>
<td>5</td>
<td>3</td>
<td>19</td>
<td>55, 56</td>
<td></td>
</tr>
<tr>
<td>Bladder cancer (papillomatosis or superficial)</td>
<td>55</td>
<td>20</td>
<td>16</td>
<td>65</td>
<td>57-59</td>
<td></td>
</tr>
<tr>
<td>Head and neck (squamous)</td>
<td>11</td>
<td>4</td>
<td>6</td>
<td>91</td>
<td>60, 61</td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal</td>
<td>13</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>15</td>
<td>62</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>43</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Hematological malignancies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hairy cell leukemia&lt;sup&gt;a&lt;/sup&gt;</td>
<td>158</td>
<td>22</td>
<td>86</td>
<td>44</td>
<td>96</td>
<td>64-71</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>107</td>
<td>12</td>
<td>37</td>
<td>6</td>
<td>46</td>
<td>74-77</td>
</tr>
<tr>
<td>Intermediate and high grade</td>
<td>61</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>15</td>
<td>74-75</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>21</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>19</td>
<td>75, 76</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma</td>
<td>42</td>
<td>8</td>
<td>14</td>
<td>3</td>
<td>50</td>
<td>78-80</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>73</td>
<td>0</td>
<td>12</td>
<td>16</td>
<td>76, 77, 81, 82, 91</td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>224</td>
<td>3</td>
<td>41&lt;sup&gt;f&lt;/sup&gt;</td>
<td>17</td>
<td>85-87</td>
<td></td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>68</td>
<td>8</td>
<td>46</td>
<td>7</td>
<td>81</td>
<td>88</td>
</tr>
</tbody>
</table>

<sup>a</sup> CR, complete response (abscence of disease); PR, partial response (>50% decrease in disease); MR, minor response (less than a partial response); % of total response, CR + PR/number of evaluable patients; AIDS, acquired immunodeficiency syndrome.
<sup>b</sup> Complete response means absence of hairy cells in the bone marrow and normalization of peripheral blood leukocytes, platelets, and erythrocytes. Partial response means a normalization of peripheral blood leukocytes, platelets, and erythrocytes and a >50% reduction in hairy cells in the bone marrow. Minor response generally means improvement in hemoglobin to more than 10 g/dl or improvement in platelets to more than 100 x 10<sup>3</sup>/liter or improvement in neutrophils to more than 1 x 10<sup>4</sup>/liter. Percentage of total response for hairy cell leukemia includes minor responses.
<sup>c</sup> Complete response and partial response not available from all trials; percentage of total response includes all responses.
low dose of α-interferon (3–4 × 10⁶ units 3 times/week) with a lower dose (0.3–0.4 × 10⁶ units 3 times/week) are under way. Preliminary results suggest efficacy for the lower dose (73). Although α-interferon should not necessarily replace splenectomy as primary therapy for hairy cell leukemia, it is useful in patients who are not surgical candidates or who have failed splenectomy.

α-Interferon is active in several other hematological malignancies. Approximately 50% of patients with low grade non-Hodgkin’s lymphoma or cutaneous T-cell lymphoma respond to α-interferon (74–80). Although it does not appear to be active in advanced chronic lymphocytic leukemia (76, 77, 81, 82, 91), results in previously untreated patients are more encouraging (83, 84). Modest activity is reported in patients with multiple myeloma (85–87). Over 80% of patients with chronic lymphocytic leukemia in the chronic phase respond to α-interferon with excellent control of blood counts (88). In a small fraction of these patients the percentage of cells with the Philadelphia chromosome decreases or disappears, albeit transiently (88). Studies are under way to demonstrate whether α-interferon prolongs the duration of chronic phase and/or survival in CML. γ-Interferon also has activity in CML (92); trials of α- and γ-interferon in CML are in progress.

Recombinant β-interferon recently entered clinical trials. Its activity seems similar to α-interferon (24). In contrast, γ-interferon has demonstrated limited antitumor activity (93–95) with the possible exception of CML (92). However, γ-interferon enhances immune responses at low doses (96).

Interleukin 2

IL-2 is a Mr 15,000 glycoprotein of 133 amino acids. IL-2 is released following antigen recognition and presentation to T-cells, and causes T-cell proliferation. It was originally referred to as T-cell growth factor. IL-2 is currently available as a recombinant molecule and has been used in many clinical trials. IL-2 causes lymphoid proliferation both in vitro and in vivo (97). It activates the lytic mechanisms of LAK cells for fresh tumors and appears to activate cytolytic T-cells (98–100). It enhances the effect of transferred LAK cells and cytolytic T-cells (101, 102). IL-2 affects the vascular endothelium and causes emigration of lymphoid cells from the blood into tissues (103, 104). IL-2 causes the release of other lymphokines including γ-interferon and TNF which likely mediate additional effects (97, 105).

IL-2 is active during the early stages of tumor growth (day 3) in both immunogenic and nonimmunogenic murine tumors (106–108). IL-2 is active in the advanced stages of tumor growth (day 10) but only in animals with weakly immunogenic tumors. The antitumor effect in nonimmunogenic tumors is mediated primarily by LAK cells and in weakly immunogenic tumors by T-cells as well as LAK cells (109).

Infusion of IL-2 in humans is associated with increases in mature T-cells (97). It also induces expression of IL-2 receptors on both T-cells and monocytes and enhances tumor cell lysis by immune effector cells (110).

IL-2 as a single agent is active in renal cell cancer, melanoma, and non-Hodgkin’s lymphoma (111). Studies are under way in other tumor types. The toxicity of IL-2 administered systemically is substantial and includes fever, chills, nausea, vomiting, diarrhea, hypotension, cutaneous erythema, fluid retention, eosinophilia, anemia, and moderate to severe hepatic and renal dysfunction (111). A limited number of patients develop neuropsychiatric complications heralded by confusion. This may last days to weeks. Most other IL-2 toxicities resolve within hours to days following discontinuation of IL-2. IL-2-activated lymphocytes adhere to endothelial cells in a dose-dependent manner. These cells are cytotoxic in vitro to endothelial cells which may explain some of the systemic toxicity (112). Increased expression of HLA-DR antigens occurs in tumor, endothelial cells, and in perivascular T-cells in patients receiving IL-2 (104). Preliminary data suggest that this may be greater in patients whose lesions respond to IL-2 treatment (109). The mechanisms by which IL-2 mediates antitumor effects are not known but histological examination of tumors before and after treatment shows infiltration with large activated T-cells consistent with a cell-mediated immune response (113).

IL-2 is known to stimulate all subsets of T-cells. A strategy was designed to decrease T-suppressor cells by injecting low-dose cyclophosphamide prior to IL-2 administration (114). Six of 24 patients with melanoma responded and all responding patients had LAK cell activation in vivo. Other strategies to improve IL-2 activity include combinations with interferons, tumor necrosis factor, or monoclonal antibodies. One preliminary study reported 6 of 21 (29%) persons with advanced renal cell carcinoma responding to the combination of IL-2 and β-interferon (115).

Tumor Necrosis Factor

TNF was first identified in mice primed with BCG and challenged with endotoxin. Serum from these mice caused tumor necrosis when transferred into tumor-bearing animals (116). This factor is produced by monocytes and is called TNF-α. Recent data indicate that cachectin, responsible for wasting in chronic parasitic diseases, is identical to TNF-α (117). A related cytotoxic protein, called lymphotixin or TNF-β, is produced by lymphocytes (118, 119). The genes for encoding both TNF-α and TNF-β have been molecularly cloned and sequenced (120–124). Both molecules have cytostatic and cytotoxic effects in vitro against a variety of human tumors (125–127). Antitumor effects have been demonstrated in syngeneic murine tumor models and a human tumor xenograft model in nude mice (128). Tumor necrosis factor has recently been studied in Phase I trials (129–132). Toxicity was over a broad range of doses; antitumor activity was minimal. Some serious toxicities such as hypertension are not dose related. Some investigators have found synergistic antitumor activities in vitro and in vivo when TNF is combined with interferon or cyclophosphamide (133–136). TNF is currently being studied with other biologicals.

Adoptive Cellular Therapy

A variety of immune cells have antitumor activity including T-cells, NK cells, killer cells, monocytes/macrophages, and polymorphonuclear leukocytes. B-cells mediate their antitumor effect by producing antibodies that combine with killer cells, monocytes, or polymorphonuclear cells and mediate antibody-dependent cellular cytotoxicity. Monocytes make TNF and peroxides which are toxic to tumor cells. Polymorphonuclear cells also generate peroxides and contain enzymes that are toxic to tumor cells. NK cells, killer cells, and cytotoxic T-cells all make lymphokines, such as natural killer cytotoxic factor, that kill or inhibit the growth of tumor cells.

The different cells that kill tumor cells recognize their targets in distinct ways. Cytotoxic T-cells recognize the tumor-associated antigen together with self-antigens. Killer cells, monocytes,
and polymorphonuclear cells mediate antibody-dependent cellular toxicity by binding of their Fc receptor to the Fc portion of the antibody attached to the target tumor cell.

The most extensively studied adoptive cellular therapy is the LAK cell described by Grimm et al. (137). They demonstrated that incubation of normal mouse splenocytes or human peripheral mononuclear cells with IL-2 in vitro for 3–5 days resulted in generation of cells capable of lysing a spectrum of fresh and cultured tumor cells in vitro. LAK cells are distinguished from NK cells because of their IL-2 dependence. LAK cells are non-major histocompatibility complex restricted, and lack mature T-cell markers (CD3) although they have other T-cell markers such as CD2. They also express the Fc and C3bi receptors (138). No efficacy or major toxicity was seen in cancer patients treated with LAK cells alone. Later studies demonstrated that lymphocytes obtained by repetitive leukapheresis, cultured with IL-2 for 3–4 days, and reinfused with IL-2 resulted in both partial and complete responses in patients with disseminated solid tumors (101). Several investigators have reported responses in patients with melanoma, renal cell carcinoma, colorectal carcinoma, and non-Hodgkin's lymphoma treated with IL-2 and LAK cells (111, 139–145) (Table 3).

There are critical questions regarding the mechanism by which IL-2 in combination with LAK cells mediates their antitumor effect. In addition, the relative roles of IL-2 and LAK cells remain to be clarified. It has not been demonstrated that the LAK cells themselves actually infiltrate the tumor or whether they are necessary in addition to the IL-2. Current trials are addressing these issues. Most of the toxicity associated with this therapy is thought to be related to increase in capillary permeability directly or indirectly related to IL-2. Recent studies using lower doses of IL-2 given by continuous infusion (3–6 milliunits/m² daily) in combination with LAK cells have reported efficacy (139). Toxicity is decreased with minimal fluid retention; few patients required intensive care. The in vivo generation of LAK cells appears to be greater following continuous infusion compared to bolus therapy (146). Trials are under way to determine the efficacy of low dose continuous infusional therapy. Other approaches to LAK cell therapy with IL-2 include i.a., i.p., and intrapleural infusions (147–151); clinical responses have been observed. However, i.p. fibrosis has been a problem following i.p. therapy (147). Other strategies to improve LAK therapy include in vitro incubation with IL-4 in addition to IL-2 which may synergize the generation of LAK cells (152) (Table 4).

Another approach to adoptive immunotherapy is to expand tumor-specific T-lymphocytes that have infiltrated the tumor in vitro. These cells are referred to as tumor-infiltrating lymphocytes and are reported to be 50 to 100 times more tumor-specific T-lymphocytes that have infiltrated the tumor (152) (Table 4).

Table 3 Clinical responses to interleukin 2 with and without lymphokine-activated killer cells

| Renal cell cancer | 131 | 9 | 32 | 31 | 111, 139–143 |
| Melanoma | 83 | 2 | 16 | 22 | 111, 139, 141, 143, 144 |
| Colorectal cancer | 26 | 1 | 2 | 12 | 111, 139 |
| Lung | 10 | 1 | 1 | 10 | 111, 139 |
| Non-Hodgkin's lymphoma | 4 | 1 | 2 | 50 | 111, 139, 141 |
| Hodgkin's disease | 4 | 0 | 3 | 75 | 138, 141 |
| Glioblastoma multiforme | 6 | 0 | 0 | 0 | 145 |

* Injected through an Ommaya reservoir.

(153–156). TIL may expand better and have higher antitumor cytotoxicity than LAK cells and may be a potentially better adoptive immunotherapy modality. TIL appear to be heterogeneous and have demonstrated both major histocompatibility-restricted (156) and nonrestricted activity (154, 155). TIL demonstrated strong cytotoxicity when tested against several allogeneic fresh tumor cells and cell lines. The actual cytotoxic cell(s) within the TIL population is controversial; although most TIL are reported to be cytolytic CD8-positive T-lymphocytes (154), the antitumor effector cells were reported to be large granular lymphocytes by others (155). Clinical trials with TIL are under way; promising initial results have been reported (157, 158). In one recent trial (158) 60% of 15 melanoma patients previously not treated with IL-2 responded to therapy with cyclophosphamide, TIL, and IL-2. Interestingly, 2 of 5 patients (40%) who had failed prior IL-2 therapy responded to this combined therapy.

A phase I clinical trial of γ-interferon-activated autologous monocytes was performed in patients with colon carcinoma limited to the peritoneal cavity (159). These investigators used countercurrent centrifugal elutriation to isolate pure preparations of peripheral blood monocytes. These cells were activated with γ-interferon and administrated to patients who had undergone an attempted curative resection of residual i.p. colon cancer. The therapy was well tolerated but efficacy is unknown.

Human Growth Factors

Hematopoietic cells are derived from self-renewing pluripotent stem cells. Pluripotent stem cells are able to differentiate into committed progenitor cells which eventually give rise to discrete cell lineages. A variety of in vitro assays are used to examine stem cells and their progeny. The least differentiated, pluripotent stem cell that can be identified in culture is the CFU (colony-forming unit)-blast (160). The CFU-GEMM (granulocytes, erythrocytes, megakaryocytes and monocytes) assay detects a progenitor cell with limited self-renewal but the capacity to generate all of the above cell types (161). More committed colony-forming stem cells have been identified. These stem cells can form erythroid, megakaryocytic and mixed granulocyte/monocyte colonies. Growth factors are essential for differentiation of all of the aforementioned progenitor cells. Some growth factors are specific for one type of progenitor cell, while others are pleiotropic and affect many types of progenitors (162,163). Growth factors are produced by many different cells (164). For instance, T cells produce IL-3 and GM-CSF. Monocytes produce M-CSF and G-CSF after contact with IL-3 and GM-CSF. Monocytes also produce IL-1 and TNF which stimulate GM-CSF, G-CSF and M-CSF production by endothelial cells. A number of growth factors can now be produced in large quantities since the complementary DNAs have been cloned (165–169) (Table 5). In Fig. 1, the hematopoietic cells responsive to each of the growth factors are shown. While a comprehensive review of growth factors is beyond the scope of this paper, this section will focus on those that have current clinical utility. IL-3 (multi-CSF) appears to be a pan-growth...
factor for all lineages. GM-CSF enhances the growth of all progenitor cells beyond the CFU-blast and is an effective enhancer of granulocyte and monocyte function. *In vitro* administration of G-CSF causes an increase in circulating neutrophils. M-CSF causes an increase in monocyte number and function.

IL-1 represents a family of polypeptides with a wide range of biological activities including augmentation of cellular immune responses (T-, B-, and NK cells); proliferation of fibroblasts; chemotaxis of monocytes, neutrophils, and lymphocytes; stimulation of prostaglandin E<sub>2</sub>, increased blood neutrophils; and neutrophil activation (170). Murine and human complementary DNAs have been cloned (171,172). IL-1 appears to synergize with other growth factors as well as having a survival-enhancing or maintenance effect on primitive hematopoietic cells (173, 174). IL-1 is known to synergize with interferon and IL-2 in enhancing tumor killing by NK cells (175).

Administration of recombinant growth factors to non-human animals and patients has been accomplished. While IL-3 has not been used in humans, injection into experimental animals activates all types of progenitor cells and induces cell cycle entry (176–178). In non-human primates, GM-CSF has been reported to cause an increase in neutrophils, eosinophils, platelets and lymphocytes (179). Recently, neutropenic patients with AIDS had an increase in neutrophil counts after receiving GM-CSF (180), and patients with metastatic sarcoma who were given recombinant GM-CSF immediately following combination chemotherapy demonstrated a significant reduction in duration and degree of neutropenia (181). GM-CSF also stimulated hematopoiesis in patients with myelodysplastic syndromes and aplastic anemia with short-term hematological improvement (182,183). Patients with melanoma and breast cancer receiving high dose chemotherapy and autologous bone marrow transplantation had an accelerated granulocyte recovery following infusions with recombinant GM-CSF (184). The toxicity of GM-CSF has been limited to low grade fever, myalgias, phlebitis, and flushing. Patients with transitional cell carcinoma of the bladder received recombinant G-CSF after the completion of combination chemotherapy and had an absolute
neutrophil count over three times higher than those not receiving G-CSF (185) without toxicity. G-CSF was also shown to reduce neutropenia caused by melphalan in patients with advanced malignancies (186). The role for M-CSF is less clear because it is not very effective in promoting in vitro growth of human monocyte progenitor cells. However, it is a potent stimulator of monocyte cytotoxicity.

There is a potential role for growth factors to decrease myelosuppression secondary to chemotherapy and/or radiation therapy. It appears likely that growth factors may allow for higher doses in situations where the major toxicity is bone marrow suppression. The increased doses may lead to better and more durable responses. Clearly, the toxicity to other organs will limit how high a dose can be given. In the setting of AML a potential problem is that the myeloid leukemia cells may proliferate in response to the growth factors (187). The growth factors may be useful for local administration into certain infected sites. Enhanced monocyte function by growth factors may be clinically useful. For example, certain monoclonal antibodies are active in monocyte-mediated, antibody-dependent cellular cytotoxicity. Combination therapy with such antibodies and M-CSF may augment tumor lysis.

Patients with pancytopenia secondary to aplastic anemia, myelodysplasia, infections, etc., may also benefit from growth factor therapy. While aplastic anemia patients could not be infused continuously with growth factors they may benefit during infections or bleeding episodes. In transient pancytopenic states growth factors may be a major benefit.

While growth factors may enhance leukemic cell growth in vitro, it is possible that appropriate growth factor(s) may lead to terminal differentiation of leukemic cells. Alternatively, the stimulation of leukemic cell proliferation may be desirable if combined with cell-cycle specific chemotherapy. Early trials with growth factors have demonstrated their potentially important clinical role. Further trials will determine the optimum dose schedules, toxicity and whether combinations of growth factors are more effective than single growth factors. This is an exciting new area of cancer research which will generate important clinical data over the next few years.

Monoclonal Antibodies

The development of technology to produce monoclonal antibodies created substantial enthusiasm for using this approach in the diagnosis and treatment of cancer. Monoclonal antibodies are specific for single antigens, can be produced in large quantities from ascites fluid or by tissue culture production techniques with high degrees of purity (greater than 90%), and can be efficiently coupled to isotopes, drugs, and toxins. The specificity of monoclonal antibodies should theoretically reduce toxicity to normal tissues unreactive with the antibody. Unlike conventional sera, there is little batch to batch variation, and they are of a single immunoglobulin subclass.

Critical Factors for Successful Monoclonal Antibody Therapy. Monoclonal antibodies (8) are useful for in vitro diagnosis particularly in leukemias and lymphomas (199). In vivo applications have evolved more slowly. Several critical factors need to be addressed (Table 6). One problem is that for in vivo use these antibodies must have minimal cross-reactivity with normal tissues. Most monoclonal antibodies cross-react with some normal tissues. This is not unexpected since these antibodies are directed against tumor-associated rather than tumor-specific antigens. To be effective these antibodies must not cross-react with antigens on normal tissues or at least the antigen density should be less than on tumors. Ideally, the antigen should be dense and homogeneous on the tumor cell surface and the antibody should bind to the antigen with high affinity. It is also important that the antigen-antibody complex not dissociate from the cell membrane although internal incorporation of the antigen-antibody complex by pinocytosis might be advantageous when immunonjugates with drugs, toxins, or isotopes are used. Some tumor-associated antigens are present in the blood. These might bind the antibody and prevent tumor localization. Ideally, one should choose antibodies selective for noncirculating antigens.

Another critical issue is the form of the antibody. Intact immunoglobulin can be broken down into various fragments. Intact IgG has a half-life of approximately 24 h, F(ab’) 10 h, and Fab 90 min. The choice of the appropriate form of antibody varies with the tumor antigen system and the conjugate. For unlabeled antibody therapy, fragments of antibody would not be useful as the Fc portion is critical for activating human effector systems.

For antibody to react with tumor cells, the tumor must be vascular. The number of blood vessels in the tumor decreases proportionately as the tumor enlarges. In addition, as the tumor enlarges, the diameter of the capillaries increases leading to a decrease in the total vascular cross-sectional area of the tumor. It is not unusual for the outer, better perfused, more viable portions of tumors to concentrate more antibodies than the less vascular, more necrotic interior portions. Other problems are arteriovenous shunts. Blood entering the tumor is then shunted to the systemic circulation before it can traverse the capillary bed, thus preventing exchange of the antibody. In addition, the low pH of tumors decreases capillary flexibility which further decreases vascular perfusion.

Human Antiglobulin Response. One major problem in clinical trials using murine monoclonal antibodies is development of human anti-mouse antibodies (antiglobulin response). These antibodies can form immune complexes and result in tissue damage. They also alter the clearance and organ distribution of injected antibodies, may neutralize the antibody, or may prevent it from binding to tumor cells.

Development of antiglobulin varies in different tumor systems (200). Patients with advanced chronic lymphocytic leukemia, who are typically hypogammaglobulinemic, rarely develop antiglobulins (201, 202). Most patients with cutaneous T-cell lymphomas and solid tumors will develop antiglobulins (200). In some tumors like melanoma, only one-third to one-half of patients developed antiglobulins (203, 204).

The specificity of the antiglobulin is important. Most antiglobulins cross-react with almost all mouse immunoglobulin classes. However, in several cases, one component of the anti-

<table>
<thead>
<tr>
<th>Table 6 Critical factors for monoclonal antibody tumor localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-reactivity with normal tissues.</td>
</tr>
<tr>
<td>Distribution of surface membrane antigens.</td>
</tr>
<tr>
<td>Affinity of antibody.</td>
</tr>
<tr>
<td>Antigenic modulation.</td>
</tr>
<tr>
<td>Circulating antigen.</td>
</tr>
<tr>
<td>Whole immunoglobulin vs. fragments.</td>
</tr>
<tr>
<td>Tumor vascularization.</td>
</tr>
<tr>
<td>Tumor size.</td>
</tr>
<tr>
<td>Degree of tumor necrosis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 7 Potential approaches to the antiglobulin problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large quantities of antibody (&gt;400 mg) may tolerate patient.</td>
</tr>
<tr>
<td>Cyclophosphamide may destroy the antiglobulin clone.</td>
</tr>
<tr>
<td>Human antibodies or chimeric human-mouse antibodies may be substituted for murine antibodies.</td>
</tr>
<tr>
<td>Fragments of immunoglobulin may reduce antigenicity.</td>
</tr>
</tbody>
</table>
globulin appeared specific for administrated antibody. This is termed an anti-idiotype. Development of anti-idiotype antibodies clearly indicates a primary immune response. Responses to other components of administered antibodies such as the Fc fragment are rapid in onset and consist primarily of IgG suggesting a secondary immune response (200).

Several putative approaches are used to prevent development of anti-globulins (Table 7). Some data suggest that the use of fragments will reduce this problem but this is controversial (205). In other studies, large quantities of antibodies (>400 mg) are used to “achieve tolerance” (206); these data are also controversial (203). In one recent study, nonspecific or unrelated monoclonal antibodies were injected just prior to the relevant monoclonal antibody (207). The objective was to increase the exogenous antibody mass and thereby render the specific antibody less immunogenic. Other approaches to prevent antiglobulins include concomitant use of immunosuppressive drugs, such as azothioprine or cyclophosphamide, given at an appropriate time to eradicate responsive clones of B-cells that would otherwise produce antiglobulin (208). Other studies use cyclosporine to interfere with T-cell stimulation of the B-cell responses. None of these approaches has been convincingly shown to be effective. Another approach might be using human antibodies or genetically engineering antibodies so that the Fc and common portions of the antibody are of human origin whereas the Fab portion is of murine origin (209–211). These chimeric antibodies offer the possibility of reduced “antigenicity.” However, these chimeric molecules would not prevent anti-idiotypic or -idiotype responses.

Clinical Trial with Unlabeled Antibodies. Clinical trials with unlabeled monoclonal antibodies, i.e., not bound to drugs, toxins, or isotopes, have resulted in only minor responses (Table 8). This was not surprising because most murine antibodies do not activate human effector systems or have direct cytotoxic effects. The most impressive clinical responses with unlabeled antibodies have been with anti-idiotypic antibodies in B-cell lymphoma patients (221, 222) and in melanoma patients treated with an antibody that activates human complement and effector cells (223).

In some studies monoclonal antibodies to regulatory molecule receptors have been studied (224, 225). Preclinical trials indicate responses in mice treated with monoclonal antibody directed to epidermal growth factor (224) and transferrin receptors (225). A trial in patients with adult T-cell leukemia treated with the anti-Tac antibody (anti-IL-2 receptor, or CD25 antigen) demonstrated a transient response in one patient; a second patient had a 6-month remission with regression of skin lesions and hematological abnormalities (220). One novel therapeutic approach involves the use of monoclonal anti-idiotype antibodies in B-cell malignancies. Unlike the aforementioned antibodies in which the antigen is a tumor-associated antigen; anti-idiotype antibodies are directed against a tumor-specific antigen; the idiotype of the cell surface immunoglobulin present on the malignant B-cells. Since B-cell lymphomas and leukemias are clonal, all of the malignant cells should express the same idiotype. Since each patients’ lymphoma idiotype is unique; anti-idiotype antibodies must be “tailor-made” for each patient.

The largest experience with anti-idiotype therapy is that of Miller et al. (224). Their initial report of this therapy was in a patient with rapidly progressive lymphoma resistant to chemotherapy and interferon. Following eight continuous 6-h i.v. infusions spaced over the period of 1 month, the patient entered a complete clinical remission that was sustained for 6 years without further treatment.3 The mechanism responsible for this is not clear. Because the antitumor response continued long
after the period of passive antibody administration, evidence of an anti-anti-idiotype antibody response by the patient was investigated; none was detected. It remains possible that indirect mechanisms may be involved. The immune system is regulated by networks of interactions between idiotypes and anti-idiotypes (226). The anti-idiotype could have triggered these interactions leading to an antiproliferative response against the tumor.

These investigators treated 10 additional patients with individually tailored anti-idiotype antibodies of different subclasses (222). Some were treated with multiple antibodies with different isotype or epitope specificity. Tumor responses were reported in 50% of patients; no complete responses were observed and most lasted for less than 6 months. Problems included anti-globulin responses and free idiotype antibody in the serum which blocked the murine antibody and lead to fever, antralgias, dyspnea, and headaches. A more serious problem was emergence of idiotype variants within tumors during treatment (227). These investigators are currently generating monoclonal anti-idiotype antibodies that identify “shared idiotypes”. Shared anti-idiotype antibodies react with the tumor cells from more than one patient. Panels of “shared idiotype” antibodies would lead to a much broader application of this novel approach to B-leukemia/lymphoma therapy.

Combination therapy with other biological response modifiers might increase the antitumor effect of monoclonal antibodies. For instance, M-CSF stimulates monocyte tumoricidal activity and phagocytosis (164). Antibodies that act through monocyte-dependent cellular cytotoxicity might have a greater effect when given with M-CSF or γ-interferon (228). IL-2 activates human killer cells (98-100) and can enhance the activity of antibodies that mediate killer cell cytotoxicity (229). IL-2 and α-interferon have been shown to synergistically enhance the effect of anti-idiotype antibodies in a transplantable murine B-cell lymphoma (230, 231). A combination of IL-2 and LAK cells incubated with an appropriate monoclonal antibody in vitro is particularly appealing. Combination trials such as those described are under way with several antibodies and biologicals.

At the turn of the century, Ehrlich coined the term “magic bullet” (232). He envisioned antibodies (at the time polyclonal antisera) delivering toxic agents directly to tumor cells to eradicate the tumor cells. This concept was repopularized with the introduction of the monoclonal antibody technology. A variety of potentially toxic molecules have been linked to monoclonal antibodies. Most of this work has been focused on the use of isotopes, toxins, and drugs coupled to antibodies. Some advantages of these immunoconjugates are reviewed in Table 9. Radioisotopes have the advantage that the radiation field can radiate beyond the antibody-binding cell. The extent of the radiation field depends on the type of isotope used (discussed below). The disadvantage is that some normal tissues may be radiated. Another advantage of radioisotopes is that it is not essential that they be internalized. Toxins and drugs require internalization to destroy the target cell.

Immunconjugates with Toxins. Several potent plant and bacterial toxins have been coupled to antibodies. Plant toxins include ricin, abrin, gelonin, pokeweed antiviral protein, and saporin. The most commonly studied bacterial toxins are diphtheria toxin and Pseudomonas exotoxin. All of these immunotoxin conjugates demonstrate specific antitumor cytotoxicity in vitro and in vivo (233–237). For many toxins a single molecule may destroy a cell. Some toxins, such as ricin, abrin, and diphtheria toxin, consist of A and B chains. The A chain is cytotoxic to the tumor cell by inhibition of protein synthesis. The B chain binds galactose which is found in high density on the surface of most cells. Entry of the A chain into cells depends on B chain binding to the cell surface. For in vivo therapy with immunotoxins it is necessary to isolate the A chain and covalently link it to the monoclonal antibody. Elimination of the B chain prevents nonspecific binding. Clinical trials of this approach are under way (238, 239). In one of these studies modest activity was reported in patients with malignant melanoma (239).

Immunconjugates with Cytotoxic Drugs. Another approach to generating immunoconjugates is by attaching cytotoxic drugs such as doxorubicin, daunorubicin, methotrexate, vinblastine, or melphalan to monoclonal antibodies (233, 240). The advantage of using drugs is that their antitumor activity and toxicity are well defined. Conjugation to antibodies allows direct targeting of the drug to tumor cells and should reduce toxicity to normal tissues. Some drugs may be more active against a tumor when delivered to the tumor cell by a monoclonal antibody. In addition, some potent drugs not used clinically because of excessive toxicity may be usable when incorporated into immunoconjugates. The disadvantage of drugs is that they are not as potent as toxins and cannot kill cells other than the single cell the antibody binds.

Antibody-coated liposomes containing chemotherapeutic agents provide an alternate method of selective drug delivery. Antibodies are either conjugated to the liposome or linked via protein A (241). Antibody-labeled liposomes containing chemotherapy drugs are demonstrated to increase cytotoxicity against murine and human tumor cell lines (242, 243). A correlation between growth inhibition and liposome internalization was demonstrated in one study. These data suggest that this approach may not be useful with antibodies that do not modulate (244).

Immunconjugates with Isotopes. The major limitation of diagnostic imaging techniques is the lack of specificity. The attractive feature of radiolabeled monoclonal antibodies is the potential to specifically localize to tumor cells. Similarly, conventional radiation therapy is not specific for tumor cells. The field that can be radiated is also limited. Radiolabeled antibodies should be able to radiate tumor deposits throughout the body with minimal radiation to normal tissues (reviewed in Refs. 245 and 246). Diagnostic. Several radionuclides are available that can be linked to monoclonal antibodies for diagnostic purposes. The choice is governed by radionuclide energy, half-life, technical requirements for conjugation, conjugate stability, safety, and cost. It is preferable to use radionuclides with energies between 100 and 200 keV which is the optimal energy for the gamma cameras currently in use (Table 10). The radionuclides should have minimal particulate radiation to maximize their safety. They should have an adequate half-life to permit tumor localization and background clearance but sufficiently short to minimize radiation exposure of the patient and medical staff.

<table>
<thead>
<tr>
<th>Table 9</th>
<th>Advantages of different immunoconjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioisotopes</td>
<td>Large variety with widely varied characteristics. Most isotopes radiate beyond a single cell. Most isotopes will not require internalization.</td>
</tr>
<tr>
<td>Toxins</td>
<td>Extremely potent. Well defined chemistry.</td>
</tr>
<tr>
<td>Drugs</td>
<td>Proven “track record” in cancer therapy. Toxicity well defined. Maximal tolerated doses known. New and more potent drugs may be useful as immunoconjugates.</td>
</tr>
</tbody>
</table>
99mTc, 111In, and 123I have suitable properties for diagnostic imaging. 131I is most often used because of its availability and low cost. However, 131I is not ideal because of high γ-ray energy that degrades images, dehalogenation in vivo; its 8-day half-life and particulate β emissions limit the total dose. Currently, 123I is too costly, is not widely available, and dehalogenates in vivo. 111In is generally conjugated to antibodies via chelation to diethyleneetriaminepentaacetic acid. Nonspecific localization in the liver and spleen has been a problem with the 111In. 99mTc would appear to be the optimal isotope, if the problems of kinetics and clearance of antibody can be overcome.

The first diagnostic imaging studies were with monoclonal antibodies using 131I-labeled anti-carcinoembryonic antigen monoclonal antibody. Background subtraction was required in order for the tumor to be imaged (247). In another study 123I-labeled antibodies that reacted with the human milk fat globule was used (248). The first systematic human study of 131I-labeled monoclonal antibody Fab fragments was reported in patients with malignant melanoma (249). Approximately 60% of the known sites were detected by scanning and more than 90% of patients had at least one site detected. Tumors with diameters <1.5 cm and those with poor antigen expression accounted for most negative findings. Significant deiodination of the protein in vivo was reported. Numerous other studies of iodinated monoclonal antibodies have been reported; all have similar problems.

There have been a number of studies using 111In-labeled antibodies. Lesions as small as 1 cm have been detected (250, 251) (Fig. 2). Problems encountered with 111In have been reticuloendothelial uptake of the antibody which has compromised imaging of lesions in the liver and spleen.

Several studies report improved localization of the radiolabeled antibody when unlabeled antibody is given simultaneously with 111In-labeled antibody (251, 252). Detection rates have increased 2- to 3-fold using this technique. Apparently the unlabeled antibody favorably affects the biodistribution of the labeled antibody and improves imaging. An irrelevant antibody that does not bind to the same site can achieve similar results.

Excellent imaging of cutaneous T-cell lymphoma patients is observed using 111In-labeled T101 antibody (250). T101 binds to circulating T-lymphocytes and the antigen-antibody complex modulates internally (253). It is believed that the circulating cells with the internalized complex traffic to the malignant lymph nodes and skin enhancing the localization at these sites. Concentrations of 0.01 to 0.03% injected dose per g were achieved in diseased lymph nodes, which is approximately 10-fold greater than previously achieved in other systems. A problem with this study was nonspecific reticuloendothelial uptake leading to localization within the liver and spleen. Some studies in animals (254) report that linking the chelate to oxidized carbohydrate decreases reticuloendothelial uptake. If confirmed in humans this could improve results with 111In conjugates.

Utilizing 111In-labeled T101, immunolymphoscintigraphy with s.c. injections intradigitally led to excellent images below the diaphragm with minimal uptake in the liver and spleen in patients with cutaneous T-cell lymphoma (255). This study reported efficient and specific antibody binding in lymph nodes nearest to the injection site with progressive uptake of remaining antibody in more distal nodes as proximal sites approach saturation. In one patient, inguinal-femoral lymph node biopsy contained over 2% of the injected dose of 111In-labeled T101 antibody at 7 days. Although this technology does not allow for a total body image, it may offer advantages by improving the quality of imaging in critical regions important for staging certain lymphoproliferative diseases.

A diamide-dimercaptide (N2S2) chelate was developed to label antibody with 99mTc (256). Using the N2S2 chelate, excellent results in imaging malignant melanoma were demonstrated using F(ab)', and Fab fragments of an anti-melanoma antibody (257). Metastases in skin, lymph node, lung, liver, brain, bone, and spleen were detected as early as 6 h after injection. In Fig. 3 lymph node and subcutaneous lesions are demonstrated. A number of these subcutaneous lesions were not detected clinically prior to the scan. There was no significant uptake in the reticuloendothelial system. Biliary and renal excretions were visualized but cleared with time. Up to 0.03% injected dose per

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life</th>
<th>γ-Energy (keV)</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>99mTc</td>
<td>6 h</td>
<td>140</td>
<td>Availability, γ-energy, inexpensive</td>
<td>Rapid kinetics, renal and gastrointestinal uptake, complex chemistry</td>
</tr>
<tr>
<td>131I</td>
<td>8 days</td>
<td>364</td>
<td>Iodine chemistry, inexpensive, available</td>
<td>Dehalogenation, high γ-ray, β-emissions</td>
</tr>
<tr>
<td>123I</td>
<td>13 h</td>
<td>159</td>
<td>Iodine chemistry</td>
<td>Dehalogenation, expensive, not available</td>
</tr>
<tr>
<td>111In</td>
<td>3 days</td>
<td>173 247</td>
<td>γ-Energy</td>
<td>Leaches off chelate, expensive, affinity for reticuloendothelial system</td>
</tr>
</tbody>
</table>

Fig. 2. Image of a patient with cutaneous T-cell lymphoma (with the Sézary syndrome) taken 48 h after the i.v. injection of 5 mCi of 111In linked to 1 mg of T101 antibody + 10 mg unlabeled T101. Full body scans show front (left) and rear (right) views demonstrating excellent localization in cutaneous disease and involved lymph nodes with nonspecific localization in the liver and spleen.
BIOLOGICAL RESPONSE MODIFIERS

Fig. 3. Image of a patient with malignant melanoma taken 8 h after the injection of 10 mCi of $^{111}$In linked to 10 mg of the Fab fragments of the NB-ML-05 antibody demonstrating multiple lymph node and s.c. nodules (arrows). Blood pooling in the heart and nonspecific localization in the sinuses are also seen.

g was observed in excised tumors. This was comparable to that reported using $^{111}$In-labeled T101 in cutaneous T-cell lymphoma (250). Approximately 80% of known lesions were detected and an equal number of not previously diagnosed lesions were found.

In summary, imaging with monoclonal antibodies is feasible. Questionable areas can be evaluated by other techniques such as computerized axial tomography or magnetic resonance imaging. Some data indicate that tumors undetectable with present techniques may be visualized with isotope-labeled monoclonal antibodies.

Therapy. Radioisotopes such as $^{32}$P and $^{131}$I have been used to treat malignancies such as polycythemia vera and metastatic thyroid carcinoma for several years (Table 11). A reasonable extension of this approach is to link isotopes to antibodies. Several isotopes that can be used include $\alpha$-emitters; low-, medium-, and high-energy $\beta$-emitters; and radionuclides that act by electron capture and/or internal conversion (Auger electrons).

Choice of the appropriate radioisotope for therapy is complex. Longer particle length radionuclides, such as $^{90}$Y and $^{188}$Re, are preferred in tumors with heterogeneous antibody binding. This should allow for destruction of nonlabeled tumor cells but might increase toxicity to normal tissues.

Shorter particle length radionuclides are preferred where there is uniform antibody binding. Electron capture or internal conversion decay radionuclides such as $^{125}$I would be optimal in such a situation; other choices include $\alpha$-emitters such as $^{211}$Astatine.

Anti-ferritin polyclonal antibody labeled with $^{131}$I (Table 12) produced responses in persons with hepatomas and Hodgkin’s disease (258, 259). Single doses as high as 150 mCi were given with acceptable hematological toxicity. Interestingly, iodinated monoclonal anti-ferritin antibody were not as effective as polyclonal antibodies. Studies in animal models demonstrate that isotope-labeled monoclonal antibodies can be effective (264). There have been relatively few reports of human studies using radiolabeled monoclonal antibodies. Responses have been reported (260) in patients treated with the anti-Lym-1 antibody labeled with $^{131}$I. Although these patients were heavily pretreated several responded. $^{131}$I-labeled T101 antibody (150–250 mCi) has been used to treat patients with cutaneous T cell lymphoma (261). Responses were observed in two patients with skin and lymph node involvement but lasted only 2 to 3 months. Myelosuppression was dose limiting. Two B-cell lymphoma patients were treated with $^{131}$I-labeled MB-1 (250 and 480 mCi) with 1 partial and 1 complete response (262). Although both had significant myelosuppression, no intervention was required.

A Phase I study of $^{131}$I-labeled Fab in patients with malignant melanoma was recently reported (249). Patients received up to 529 mCi of $^{131}$I in divided doses; target organ toxicity was bone marrow associated. Despite good antibody localization in selected patients with estimated tumor doses of 1300–5500 cGy, anti-tumor effects were modest.

Therapy with $^{131}$I-labeled anti-ovarian antibodies i.p. demonstrated responses in 9 of 24 patients (263). Doses >140 mCi were more effective than lower doses. Only patients with small volume (<2 cm) disease responded.

The first isotope other than $^{131}$I to enter clinical trials was $^{90}$Y. $^{90}$Y has a more energetic $\beta$-particle than $^{131}$I (2.2 versus 0.6 MeV). It is bound by diethylentriaminepentaacetic acid but it has no imagible $\gamma$-ray. Rhenium has two different $\beta$-emitting isotopes ($^{186}$Re, $^{188}$Re) that are chelated by the same $\text{N}_2\text{S}_2$ ligand used for $^{99m}$Tc. Rhenium is bound irreversibly to $\text{N}_2\text{S}_2$ as is $^{99m}$Tc. Both isotopes have imagible $\gamma$-ray energies. Clinical trials with rhenium are expected to commence in 1988.

The next 2 years should see the first systematic studies of targeting radiation therapy directly to metastatic carcinoma with monoclonal antibodies. Issues such as single dose versus

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-life</th>
<th>Decay mode</th>
<th>Particulate maximum energy (MeV)</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{186}$Re</td>
<td>17 h</td>
<td>$\beta$</td>
<td>2.1</td>
<td>Generator produced, $^{99m}$Tc chemistry</td>
<td>Very energetic $\beta$-particle</td>
</tr>
<tr>
<td>$^{188}$Re</td>
<td>3.5 days</td>
<td>$\beta$</td>
<td>1.1</td>
<td>$^{99m}$Tc chemistry</td>
<td>Availability, low specific activity</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>8 days</td>
<td>$\beta$</td>
<td>0.6</td>
<td>Iodine chemistry, inexpensive</td>
<td>Dehalogenation, abundant $\gamma$-emission, half-life</td>
</tr>
<tr>
<td>$^{67}$Cu</td>
<td>2.5 days</td>
<td>$\beta$</td>
<td>0.4–0.6</td>
<td>Images</td>
<td>Availability, cost</td>
</tr>
<tr>
<td>$^{90}$Y</td>
<td>2.5 days</td>
<td>$\beta$</td>
<td>2.3</td>
<td>Indium chemistry</td>
<td>No imaging, leaks off chelate</td>
</tr>
<tr>
<td>$^{211}$Astatine</td>
<td>7 h</td>
<td>$\alpha$</td>
<td>5.9</td>
<td>Iodine chemistry, short range</td>
<td>Dehalogenation</td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>2.5 days</td>
<td>Electron capture (Auger)</td>
<td>5.7</td>
<td>Iodine chemistry</td>
<td>No imaging, dehalogenation, availability</td>
</tr>
</tbody>
</table>

Table 11 Isotopes for monoclonal antibody therapy

Downloaded from cancerres.aacrjournals.org on October 17, 2017. © 1989 American Association for Cancer Research.
Tumor Vaccines

Another potential antitumor strategy is activation of host defenses against tumor-associated antigens. This is referred to as active specific immunotherapy which attempts to boost the host's immune response. The tumor-associated antigens must be accessible on the tumor cell to serve as a site for antibody-mediated or cell-mediated destruction. Most studies of active specific immunotherapy in humans with hematological malignancies have been disappointing. For example, early studies in ALL suggested that children could be successfully immunized with allogeneic leukemia cells (3). These results could not be reproduced in controlled trials (268, 269). Similarly, early results in acute myelogenous leukemia patients suggested prolonged survival with BCG and/or allogeneic leukemia cell (4, 5, 270–272) immunization. Again, these results could not be reproduced (7, 273).

Great promise was held for BCG immunotherapy with or without cellular vaccines for malignant melanoma (2); long-term results were disappointing. However, a series of interesting studies focused on melanoma patients demonstrated active immunity in patients with promising preliminary clinical results (Table 13) (274–280).

Clinical vaccine trials for other solid tumors are limited in number. In one trial patients with colorectal cancer with transmural extension of tumor or nodal metastasis were randomized into groups treated by resection alone or resection with active specific immunotherapy with autologous tumor cells plus BCG. At 28 months follow-up recurrences were significantly fewer in vaccinated patients and they demonstrated cellular immune response to autologous tumor cells (281, 282). In a metastatic renal cell vaccine study, 4 of 14 patients responded to vaccine treatment with autologous tumor cells mixed with Corynebacterium parvum (283). In another study patients with bronchogenic carcinoma received a vaccine of allogeneic tumor cells in complete Freund's adjuvant. A 10-year follow-up demonstrated a statistically significant 5-year survival advantage for patients treated with specific active immunotherapy (284).

A major problem with tumor vaccines is our inability to standardize the contents of a vaccine, particularly with reference to the tumor-associated antigen(s) of interest. Perhaps the antiidiotypic vaccine approach discussed below will adequately address this problem.

Anti-Idiotype Vaccines

Another novel approach to tumor vaccines based on the idiotypic network hypothesis warrants discussion (226). Tumor-associated antigens are often a part of "self" and evoke a very poor immune response in the tumor-bearing host because of T-

<table>
<thead>
<tr>
<th>Disease</th>
<th>Antibody/class</th>
<th>No. of patients</th>
<th>Toxicity</th>
<th>Effect</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatoma*</td>
<td>Antiferritin/heteroantisera</td>
<td>105</td>
<td>Thrombocytopenia</td>
<td>7% complete and 41% partial responses</td>
<td>258</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>Antiferritin/heteroantisera</td>
<td>38</td>
<td>Myelosuppression</td>
<td>40% partial responses</td>
<td>259</td>
</tr>
<tr>
<td>B-lymphoma and B-CLL</td>
<td>Lym-1/1gG2a</td>
<td>18</td>
<td>Fever, rash, myelosuppression</td>
<td>2 complete and 7 partial responses</td>
<td>260</td>
</tr>
<tr>
<td>CTCL*</td>
<td>T101/1gG2a (anti-CD5)</td>
<td>6</td>
<td>Dyspnea, fever, urticaria, myelosuppression</td>
<td>2 partial, and 3 minor responses</td>
<td>261</td>
</tr>
<tr>
<td>B-Lymphoma</td>
<td>MB-1/1gG1</td>
<td>2</td>
<td>Myelosuppression</td>
<td>1 complete and 1 partial response</td>
<td>262</td>
</tr>
<tr>
<td>Melanoma</td>
<td>8.2/1gG1, 96.5/1gG2a</td>
<td>24</td>
<td>Myelosuppression, chills, fever, hypotension</td>
<td>No responses</td>
<td>249</td>
</tr>
<tr>
<td>Ovarian</td>
<td>HMFG1, HMFG2, AUAI, H17E2/1gG1</td>
<td>7</td>
<td>Myelosuppression, fever, diarrhea</td>
<td>9 partial responses</td>
<td>263</td>
</tr>
</tbody>
</table>

* Some patients were also treated with external beam irradiation and chemotherapy.
* B-CLL, B-chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma.
* Fab fragments were used in this study.
* i.p. therapy.

A major problem with tumor vaccines is our inability to standardize the contents of a vaccine, particularly with reference to the tumor-associated antigen(s) of interest. Perhaps the antiidiotypic vaccine approach discussed below will adequately address this problem.

Tumor Vaccines

Another potential antitumor strategy is activation of host defenses against tumor-associated antigens. This is referred to as active specific immunotherapy which attempts to boost the
cell-mediated suppression (285, 286) and tolerance to the antigen (287). Recent data indicate that a weak antigen can be turned into a strong antigen by simply changing the molecular environment of the haptenic structure. These changes in the hapten carrier activate T-cell help and increase the immune response. In addition, altering the carrier can turn a tolerogenic antigen into an immunogenic antigen. The immune status of cancer patient is often suppressed and they can respond to only certain T-dependent antigens but not to other antigen forms. These considerations suggest introduction of molecular changes into the tumor antigens before using them as vaccines. Presently, this is impossible to accomplish because tumor antigens are not well defined and are difficult to purify.

According to the idiotypic network hypothesis, certain anti-idiotypic antibodies express three dimensional shapes which resemble the structure of external antigens. The existence of such internal antigenic idiotypes comes as a statistical necessity of the diverse and “complete” network of idiotypes (288, 289). The internal antigens are stereochemical copies of nominal antigens which produce specific immune responses similar to responses induced by nominal antigen and also can compete with normal antigens in binding assays.

Assuming one is aiming for a tumor-specific immunotherapy, the anti-idiotypic hybridoma route of making surrogate antigens could alleviate the problem of making a large amount of purified antigen associated with a given tumor. Furthermore, idiotypetype-based tumor antigens are free of the danger of transmitting human tumor viruses which theoretically may contaminate cell-based human tumor vaccines. Parenthetically, it makes no difference in this approach whether a de novo tumor antigen is the target for immunotherapy or whether the target is a “normal” but highly tumor-associated antigen. If a tumor-specific hybridoma is available then a second anti-hybridoma (anti-idiotypic hybridoma) can be made and selected for the structure which represents the tumor antigen. In Fig. 4, this approach is diagrammed. An anti-tumor-associated antibody (Ab1) is generated and is used to make a second antibody or an anti-idiotypic antibody (Ab2).

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Stage</th>
<th>Vaccine</th>
<th>Active immunity</th>
<th>Clinical results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>III</td>
<td>Autologous with melanoma cells with BCG plus cyclophosphamide</td>
<td>Reduction in CD4+ CD45+ T-cells*</td>
<td>NR*</td>
<td>274</td>
</tr>
<tr>
<td>55</td>
<td>II and III</td>
<td>Polyvalent melanoma antigen</td>
<td>69% stage II</td>
<td>NR</td>
<td>275</td>
</tr>
<tr>
<td>39</td>
<td>I and II</td>
<td>Vaccinia melanoma onco-lysate</td>
<td>53% stage III 64%</td>
<td>Increased survival</td>
<td>276</td>
</tr>
<tr>
<td>10</td>
<td>III</td>
<td>Semiautologous melanoma hybrids with C. parvum</td>
<td>Positive delayed hypersensitivity</td>
<td>No responses</td>
<td>277</td>
</tr>
<tr>
<td>19</td>
<td>III</td>
<td>Autologous melanoma cell (± cyclophosphamide)</td>
<td>88% with cyclophosphamide; 29% without cyclophosphamide</td>
<td>2 complete responses</td>
<td>278</td>
</tr>
<tr>
<td>17</td>
<td>II</td>
<td>Allogeneic melanoma lines with BCG or C. parvum</td>
<td>94% developed antibodies to alloantigens</td>
<td>NR</td>
<td>279</td>
</tr>
<tr>
<td>26</td>
<td>Allogeneic melanoma cell lines</td>
<td>15% developed anti-GP2 antibodies. 38% developed anti-GM2 antibodies</td>
<td>NR</td>
<td>280</td>
<td></td>
</tr>
</tbody>
</table>

* NR, not reported.

\[\text{Immunize Mouse with Tumor Cell to Generate Ab1} \]
\[\text{Immunize Mouse with Ab1 to Generate Anti-Idiotypic (Ab2)} \]
\[\text{Immunize Patients with Anti-Idiotypic (Subcutaneous with Adjunct and/or Carrier)} \]

Fig. 4. Idiotype tumor network. Concept of monoclonal anti-idiotypic vaccines. An Ab1 is generated to a tumor-associated antigen. The Ab1 is used to immunize mice to generate an anti-idiotypic monoclonal Ab2. The Ab2 which is the mirror image of the tumor-associated antigen is then used to immunize patients to generate an Ab3 response to the tumor-associated antigen. Note that the Ab3 (or Ab1') has a binding specificity identical to that of the Ab1.

The cyclic nature of complementary binding sites and idiotopes serves as the basis for the idiotype vaccines. The experimental evidence that idiotypes or anti-idiotypes mimic antigens and produce antitumor immunity is quite strong (288, 291–293). Treatment of mice with injection of anti-idiotypic that appears to mimic tumor antigen structures has been reported to induce antitumor immunity in human melanoma (294), mammary adenocarcinoma (295), and rat sarcoma (296). Recently, anti-idiotypes produced in goats that contained antibody components that mimic human gastrointestinal tumor-associated antigens have been generated. Mice and rabbits immunized with this anti-idiotypic produced antibodies (Ab3) that bound to human tumor antigen (297). Monoclonal anti-idiotypic to a leukemia/lymphoma T-cell-associated antigen have evoked an Ab3 response in animals (298, 299). These Ab3 anti-idiotypic bound to the tumor antigen and immunoprecipitated an antigen identical to the Ab1. In a clinical trial where patients were given i.v. injections of murine antitumor antibodies, those patients that developed an anti-idiotypic response improved clinically and had longer remissions from their disease (300).
Indirectly, these results appeared to be consistent with the anti-idiotypic hypothesis. They did not demonstrate Ab3 responses in these patients, however. In another clinical trial (301), patients with colorectal carcinoma were immunized with goat anti-idiotypic antibodies to murine monoclonal antibody CD171A (Ab1). All patients developed Ab3 with binding specificities on the surfaces of tumor cells similar to the Ab1 specificit. The Ab3 also inhibited binding of Ab2 to Ab1 suggesting shared idiotopes with Ab1. Three of 30 treated patients responded. In another clinical trial 15 patients with disseminated melanoma were immunized with monoclonal anti-idiotypic antibodies to a high molecular weight melanoma-associated antigen (302). Seven patients developed antibodies (Ab3) which inhibited the binding of anti-idiotypic antibody (Ab2) to the Ab1 antibody and 3 patients showed tumor reduction. Current clinical trials are ongoing to further study the effects of anti-idiotypic vaccine.

Conclusion

Biological therapy may be considered the “Fourth Modality of Cancer Treatment” (321). The past decade of research has demonstrated that there is indeed a role for biological agents in cancer therapy. Over the next decade, combination therapies, new cloned molecules, and more sophisticated chemistry for immunoconjugates should lead to major advances in cancer diagnosis and therapy.

Acknowledgments

I am indebted to Drs. R. P. Gale, M. Goldrosen, L. Vaickus, and W. Biddle for their careful review of this manuscript, NeoRx Corporation for the 99mTc scan, and Pat Alvarado for the preparation of this manuscript.

Immunomodulation

Immunomodulation is a rapidly expanding field and is applicable in a variety of clinical settings including autoimmune diseases, graft rejection, as well as the treatment of cancer. Immunomodulation with lymphokines such as interferon and IL-2 have been discussed previously, and the use of BCG, Corynebacterium parvarum, and other stimulants of the immune system was also briefly reviewed. There exists a growing list of agents such as mitoxantrone (304, 305) and doxorubicin (306) that have immunomodulatory activity. Cyclophosphamide, in relatively low doses, has an anti-suppressor T-cell effect. Cyclophosphamide has been used to enhance the response to IL-2 (114) and tumor vaccines (274, 278) and also as a single agent. In one melanoma study, cyclophosphamide depression of suppressor T-cells was restricted to those patients who demonstrated increased suppressor T-cell activity prior to cyclophosphamide treatment (303). Other cytokotic agents such as mitoxantrone (304, 305) and doxorubicin (306) also have immunomodulatory activity. Corticosteroids have a profound suppressive effect on both humoral and cellular immunity and are used to treat autoimmune diseases and graft rejection. They are also lympholytic and are used to treat a variety of lymphoid neoplasms. Diethylstilbestrol (307) and the antiestrogen tamoxifen (308) also have immunomodulatory activity. Cyclosporin has been shown to have suppressor effects on both humoral and cellular arms of the immune response (309). Nonsteroidal antiinflammatory agents (310), morphine (311), marijuana (311), and antibiotics such as amikacin and cefotaxime (312) also have immunomodulatory activity. A variety of synthetic agents modulate the immune system such as polyribonucleotides which augment natural killer activity and induce interferon production (313). Thymosin fraction 5 acts as an immunostimulant and has a significant antitumor effect in animal models (314). Removal of IgG and circulating immune complexes by passing plasma over a protein A column may also modulate the immune system. Responses have been reported in a variety of cancer patients (315). Bacterial cell products such as Bordetella pertussis (316), Corynebacterium granulosum (317), and Listeria monocytogenes (318) and many others have potent immunomodulatory activity.

Another innovative approach to immunomodulation has been with monoclonal antibodies. The use of monoclonal antibodies to remove T-cells prior to allogeneic bone marrow transplant has been discussed previously (188–190). Monoclonal antibodies can also be utilized systemically to alter cell populations such as T-cells or T-cell subsets (319, 320).

References


26. Caparros, B., Rosen, G., and Cunningham-Rundles, S. Phase II trial of


102. Lotze, M. T., Custer, M. C., Sharrow, S. O., et al. In vivo administration of


200. Schroff, R. W., and Stevenson, H. C. Human immune responses to murine
202. Dillman, R. O. Shawler, D. L., Dillman, J. B., and Roystan, I. Therapy of
194. Weisbart, R. H., Kwan, L., Golde, D. W., and Gasson, J. C. Human GM-
209. Liu, A. Y., Robinson, R. R., Hellström, K. E., Murray, E. D., Jr., Chang,
197. Fletcher, M. P., and Gasson, J. C. Enhancement of neutrophil function by
granulocyte-macrophage-colony-stimulating factor involves recruitment of
3 stimulate monocyte cytotoxicity through a tumor necrosis factor-dependant
assay for detection of human monoclonal antibodies and human mono-
clonal antibody immunotherapy in patients with gastrointestinal ade-
208. Kozlowski, D. Antibody-directed liposomes: comparison of various ligands
207. Weiss, D. J., Putnam, I., Hellström, I. Chimeric mouse-human IgGl antibody that can
205. Dillman, R. O., Shawler, D. L., Dillman, J. B., and Roystan, I. Therapy of
chronic lymphocytic leukemia and cutaneous T cell lymphoma with T101
204. Schreiber, R. D., Beatty, S. M., Weisbart, R. H., et al. Human anti-
203. Dillman, R. O., Shawler, D. L., Dillman, J. B., and Roystan, I. Therapy of
chronic lymphocytic leukemia and cutaneous T cell lymphoma with T101
202. Dillman, R. O. Shawler, D. L., Dillman, J. B., and Roystan, I. Therapy of
200. Schroff, R. W., and Stevenson, H. C. Human immune responses to murine
monoclonal antibodies. In: K. A. Foon and A. C. Morgan (eds.), Monoclonal
antibody therapy in malignant melanoma: factors effecting in vivo localiza-
208. Kozlowski, D. Antibody-directed liposomes: comparison of various ligands
207. Weiss, D. J., Putnam, I., Hellström, I. Chimeric mouse-human IgGl antibody that can
205. Dillman, R. O., Shawler, D. L., Dillman, J. B., and Roystan, I. Therapy of
chronic lymphocytic leukemia and cutaneous T cell lymphoma with T101
204. Schreiber, R. D., Beatty, S. M., Weisbart, R. H., et al. Human anti-
203. Dillman, R. O., Shawler, D. L., Dillman, J. B., and Roystan, I. Therapy of
chronic lymphocytic leukemia and cutaneous T cell lymphoma with T101


Biological Response Modifiers: The New Immunotherapy

Kenneth A. Foon


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/49/7/1621.citation

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.