Utilization of Monoclonal Antibodies in the Treatment of Leukemia and Lymphoma

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The generation of murine monoclonal antibodies reactive with human leukemia and lymphoma cells has recently led to clinical trials that have begun to evaluate the use of these reagents in the treatment of various leukemias and lymphomas. Several of these studies have demonstrated that infusion of monoclonal antibody can cause the rapid and specific clearance of leukemic cells from the peripheral blood. Intravenously administered antibody also rapidly binds to bone marrow lymphoblasts, and in one instance, has resulted in the partial regression of tumor cell infiltrates in lymph nodes and skin. Unfortunately, clinically significant responses have not in general been achieved, but these clinical studies have identified specific factors that result in the development of resistance to antibody-mediated lysis in vivo. These factors include the presence of circulating antigen, antigenic modulation, reactivity of monoclonal antibody with normal cells, immune response to murine antibody, and the inefficiency of natural immune effecter mechanisms. Current research is now being directed towards developing methods to circumvent each of these obstacles. Future clinical studies utilizing antibodies in vitro or with different specificity may demonstrate greater therapeutic efficacy. In addition, monoclonal antibodies can be used as carriers of other cytotoxic agents and in conjunction with other agents that will reduce the total tumor load. Monoclonal antibodies represent new and powerful reagents that may in the near future become an additional therapeutic modality for patients with malignant disease.

IN 1900 PAUL EHRLICH wrote, “By injecting one animal with the cells of another, we can produce substances in the serum of the first, which have a specific damaging or destructive influence on these cells. This possibility has within a short time extended the theoretical doctrines of immunity in various directions.” One of these directions has been the passive administration of antibodies in the treatment of cancer. Unfortunately, even though there have been instances in which passive serotherapy has had a definite antitumor effect, these responses have been transient and have often been associated with severe systemic side effects. The major factors limiting the use of serotherapy have been: (1) the difficulty in preparing antisera with desired specificity; (2) the relatively low titer of these reagents; and (3) the inability to produce and administer the large quantities of specific antisera that would be necessary for clinical use. Within the last few years, it has been shown that each of these problems can be resolved through the generation of hybridoma antibodies to human tumor cells.

Utilizing the method developed by Kohler and Milstein, hybridoma antibodies have been generated to a wide variety of surface antigens on both normal and malignant human cells. Whereas conventional heteroantisera require extensive absorption with various human tissues before specificity for certain cells can be demonstrated, hybridoma antibodies are monoclonal and therefore have unique specificity that does not require any absorption. Since these monoclonal antibodies are the products of continuously proliferating cells, virtually unlimited amounts of antibody can be produced. Furthermore, monoclonal antibodies can be readily purified, and the clinical use of these reagents therefore does not necessitate the administration of large amounts of extraneous foreign proteins.
Since the availability of monoclonal antibodies reactive with human tumors therefore circumvents the major limitations encountered in previous serotherapy trials, there is now renewed interest in the passive administration of antibody in the treatment of cancer. Indeed, several clinical studies have recently been published, and the results of these studies demonstrate the potential usefulness of this type of specific immunotherapy. However, these clinical trials also point out further limitations of this approach that will have to be resolved before monoclonal antibodies can become a useful adjunct to current therapies of cancer. The present review focuses on those studies that have utilized monoclonal antibodies in the treatment of human leukemia and lymphoma. Although it is likely that the use of monoclonal antibodies in the treatment of solid tumors will differ from their use in hematologic tumors, the problems that have been encountered in the treatment of leukemia and lymphoma will also be relevant to the treatment of these other malignancies.

SEROTHERAPY OF EXPERIMENTAL TUMORS

Numerous previous studies have demonstrated the efficacy of passive administration of antibodies in the treatment of selected animal tumors. In these studies, it has been shown that monoclonal antibodies can be effective against relatively large numbers of tumor cells. Recently, investigators have begun to utilize monoclonal antibodies in experimental animal models to determine the effectiveness of these new reagents in serotherapy and have shown that passive administration of monoclonal antibody can be curative in selected situations. Bernstein et al. have used murine monoclonal antibodies specific for either virally induced surface antigens, normal differentiation antigens, or idiotypic tumor antigens. In these studies, serotherapy has been most effective when large amounts of antibody are given and when relatively small numbers of tumor cells are present. Very few animal studies have demonstrated effectiveness against relatively large numbers of tumor cells.

Recently, investigators have begun to utilize monoclonal antibodies in experimental animal models to determine the effectiveness of these new reagents in serotherapy and have shown that passive administration of monoclonal antibody can be curative in selected situations. Bernstein et al. have used murine monoclonal antibodies specific for a T-cell differentiation antigen (Thy-1.1) in the treatment of a transplantable murine T-cell leukemia (AKR-SL2). In one series of experiments, mice received a subcutaneous injection of 10^6 leukemia cells. Intravenous treatment with monoclonal antibody was started within 2 hr of tumor inoculation and was continued for 1 wk. Mice who received antibody infusions had a prolonged survival when compared to untreated animals, but all mice eventually died of progressive leukemia. Prolonged survival was primarily due to a delay in the onset of systemic disease, while growth of local tumors was unaffected. Serotherapy was more effective when exogenous complement (rabbit serum) was given in addition to monoclonal antibody and in these experiments, 3 of 8 animals did not develop either local or systemic disease. Even more dramatic effects were seen when the tumor inoculum was reduced tenfold (to 10^6 AKR cells), and all animals treated with both monoclonal antibody and exogenous complement were then cured of leukemia.

Further studies have attempted to define the role of antibody isotype and complement in serotherapy. Although IgG2a antibody appeared to eradicate tumor cells through complement-mediated lysis, it was found that monoclonal IgM antibody specific for Thy-1.1 was totally ineffective in vivo. The same IgM antibody and complement, however, could easily lyse AKR tumor cells in vitro. IgM antibody was not active in antibody-mediated cellular cytotoxicity (ADCC), while IgG2a antibodies could lyse tumor cells via this mechanism. Thus, the exact mechanism of tumor cell lysis has not yet been determined, and some experiments suggest that different effector mechanisms are active in different locations, i.e., circulating leukemia cells may be cleared by one mechanism while solid tumors respond to another.

The effect of antibody treatment on the host’s immune system is another important factor that has been evaluated in this animal model. Normal T lymphocytes as well as tumor cells express Thy-1.1 antigen, and it was shown that normal T cells in the thymus and lymph node were depleted following treatment with monoclonal antibody and complement. There was also a marked decrease in T-cell function in vitro. Regardless of the effect of serotherapy on normal immune function, it is also apparent that the presence of normal cells that express Thy-1.1 antigen will inhibit lysis of tumor cells since antibody has no inherent way of distinguishing normal T cells from AKR leukemia cells. Thus, effective treatment of AKR leukemia cells in this experimental model is necessarily limited by (1) the immunosuppressive effects of infusion of anti-Thy-1.1 and complement and (2) the competition of normal Thy-1.1-positive cells with AKR leukemic cells for lysis by the host’s effector mechanisms. Despite these obstacles, monoclonal antibody is in fact capable of eradicating a lethal inoculum of tumor cells, demonstrating the potential utility of this type of specific immunotherapy.

In more recent studies, Kirch and Hammerling have evaluated passive immunotherapy of murine leukemia in a model in which the monoclonal antibody was specific for tumor cells and did not react with normal host cells. They found that IgG2a and IgG3 antibodies were more effective than IgM antibodies, especially if serotherapy was initiated within 24 hr of
tumor inoculation. In one instance, however, IgG2a antibody treatment appeared to enhance tumor growth. Tumor enhancement appeared to be an inherent property of one transplantable tumor, since the same antibody was effective in the treatment of a different tumor in the same host.

**CLINICAL STUDIES UTILIZING MONOCLONAL ANTIBODIES**

At the present time, the results of several clinical studies using monoclonal antibodies in leukemia and lymphoma have been reported. Each study has dealt with a different type of leukemia or lymphoma and has utilized monoclonal antibodies with different specificities. Nevertheless, the results of these trials are similar in many respects. This is primarily evident when comparing the therapeutic responses to antibody infusion as well as in the identification of factors that have limited the effectiveness of serotherapy.

**Diffuse Poorly Differentiated Lymphocytic Lymphoma**

In 1980, Nadler et al. reported the results of serotherapy of a patient (N.B.) with a monoclonal antibody directed against a human lymphoma-associated antigen. This antibody (Ab89) was generated following immunization with patient N.B. tumor cells and was found to react with lymphoma cells from approximately 10% of patients with diffuse poorly differentiated lymphocytic lymphoma (D-PDL) and B-cell chronic lymphocytic leukemia (CLL). Ab89 did not react with normal hematopoietic cells from peripheral blood, bone marrow, tonsil, lymph node, spleen, or thymus. In vitro characterization of Ab89 showed that this IgG2a antibody-mediated complement-dependent lysis and macrophage adherence but failed to initiate antibody-dependent cell-mediated cytotoxicity (ADCC).

At the time of serotherapy, there was a very large tumor burden that included hepatomegaly, lymphadenopathy, extensive bone marrow infiltration, and an elevated WBC with approximately 400,000 tumor cells/mm³. Immediately following infusion of Ab89, there were transient reductions in numbers of circulating tumor cells on 3 of 4 occasions, which returned to pretreatment levels within 24 hr. Despite intravenous infusion of up to 1.5 g antibody, all antigenic sites on circulating lymphoma cells were not saturated with antibody. In vitro studies showed that the patient’s plasma contained large amounts of a circulating antigen that blocked the binding of Ab89 to tumor cells in vivo. Even though large amounts of Ab89 were administered and circulating immune complexes were formed, there was minimal renal toxicity and only transient reductions in creatinine clearance were noted. No other adverse effects of monoclonal antibody infusion were seen in this patient.

**Non-T Acute Lymphoblastic Leukemia**

Monoclonal antibody therapy has also been evaluated in acute lymphoblastic leukemia (ALL). These studies have utilized a monoclonal antibody (J5) specific for the common acute lymphoblastic leukemia antigen (CALLA) that is expressed by leukemic cells from 80% of patients with non-T-cell ALL and 40% of patients with chronic myelocytic leukemia in blast crisis. In addition, tumor cells from patients with T-cell lymphoblastic lymphoma, Burkitt’s lymphoma, and nodular poorly differentiated lymphocytic lymphoma have also been found to express CALLA. CALLA is not expressed by normal lymphoid cells in peripheral blood, spleen, thymus, or lymph node but is expressed by a small fraction of cells in either normal or regenerating bone marrow.

Four patients with relapsed CALLA-positive ALL received multiple infusions of J5 monoclonal antibody (IgG2a). Three patients had circulating tumor cells and, in each of these patients, there were dramatic reductions in numbers of circulating leukemic cells that began immediately after antibody infusion. Circulating lymphoblasts were not completely eliminated in these patients, however, and the recovery of these cells in the peripheral blood was often just as dramatic as their initial clearance. There was no evidence of elimination of bone marrow lymphoblasts in any of the four patients.

Phenotypic analysis of leukemic cells during antibody infusions demonstrated that J5 antibody rapidly bound to peripheral blood and bone marrow lymphoblasts and that all antigenic sites were saturated with monoclonal antibody. Furthermore, C3 was deposited on the surface of leukemic cells as a result of antibody binding. The rapid decreases in numbers of circulating lymphoblasts that were seen during serotherapy appeared to be due to the clearance of these J5 antibody and C3-coated cells. It is unlikely that these cells were directly lysed in vivo, however, since activation of human complement in vitro did not result in significant cytotoxicity.

Phenotypic studies during the course of serotherapy also demonstrated marked changes in the expression of CALLA on the cell surface of leukemic cells, which appeared to be a direct result of J5 antibody binding in vivo. Figure 1 compares the cell surface phenotype of bone marrow mononuclear cells taken before serotherapy with cells obtained 2 days after starting J5 anti-
Bone Marrow Mononuclear Cells

A. Pretreatment

B. Day 2

Fluorescence Intensity

Cell Number

Figure 1. Cell surface phenotype of bone marrow lymphoblasts during serotherapy with J5 monoclonal antibody (patient 1, ref. 35). Samples were obtained prior to treatment (A) and 48 hr after starting antibody infusion (B). Mononuclear cells were characterized by indirect immunofluorescence and analyzed on the fluorescence activated cell sorter. FACS histograms indicate reactivity with JO (negative control antibody) (-----), J5 (--), and anti-la (---).

body infusions in the first patient who was treated. As shown in these fluorescence activated cell sorter (FACS) histograms, prior to serotherapy, leukemic cells expressed both CALLA and Ia antigen (Fig. 1A). In contrast, cells obtained 48 hr later expressed only Ia antigen (Fig. 1B). In both instances, greater than 80% of the bone marrow cells were lymphoblasts by morphological criteria. Furthermore, the change in CALLA expression could not be explained by “masking” of CALLA by J5 antibody in vivo, since very little murine antibody was detectable on these cells at this time. Following serotherapy, leukemic cells in peripheral blood and bone marrow reexpressed CALLA, and the phenotype of the leukemic cells reverted to their original pattern.

In vitro studies with leukemic cells and with CALLA-positive cell lines demonstrated that the loss of CALLA was not due to emergence of a population of CALLA-negative lymphoblasts, but was rather the result of modulation of the cell surface expression of this antigen.66 CALLA modulation was directly induced by the binding of J5 antibody and was totally reversible since CALLA was reexpressed when J5 antibody was no longer present. It was, therefore, felt that resistance to serotherapy was, at least in part, due to the antigenic modulation of leukemic cells, since this resulted in the conversion of CALLA-positive cells to CALLA-negative cells that would no longer bind either J5 antibody or C3.

T-Cell Leukemias and Lymphomas

A monoclonal antibody (L17F12) specific for a 67,000 dalton protein expressed by both normal and malignant T cells56 has been used to treat adult patients with T-cell leukemias and cutaneous T-cell lymphomas. The detailed experience with two patients has recently been reported.37,38 In several respects, this clinical situation is similar to the experimental model discussed previously, which used anti-Thy-1.1 antibodies to treat a transplantable T-cell leukemia.51 There are major differences, however, in that relatively small numbers of tumor cells were present in the murine model, while the clinical trial described by Miller et al.37 necessarily involved the treatment of patients with large numbers of tumor cells in peripheral blood, bone marrow, lymphoid organs, and skin.

One patient treated with L17F12 was a 67-yr-old male with T-cell leukemia. Prior to serotherapy, this patient underwent several leukapheresis procedures with removal of between 2.3 x 10^11 and 8.3 x 10^11 cells on each occasion. A marked drop in WBC was noted with each procedure, but despite the removal of a large number of lymphoblasts with each leukapheresis, the number of circulating blasts returned to pretreatment levels within 36 hr. This patient then received 3 infusions of L17F12 antibody and the response on 2 of these occasions was similar to that seen after leukapheresis. This suggested that infusion of 1 mg of antibody resulted in the in vivo elimination of approximately 2 x 10^11 tumor cells. Although this can be considered a substantial reduction in number of tumor cells, it actually comprised a small fraction of the total leukemic cell burden that was present at the time of serotherapy.

Even though there was a transient response, L17F12 antibody was not detected on leukemic cells in vivo or as free antibody in the patient’s sera. It is not known whether C3 was deposited on leukemic cells in vivo. The patient remained asymptomatic during antibody infusions but was noted to have a transient
decrease in creatinine clearance and mild elevations of SGOT and alkaline phosphatase during serotherapy. The second reported case of serotherapy with L17F12 antibody was a 67-yr-old male with cutaneous T-cell lymphoma.\textsuperscript{38} He received 17 infusions of antibody during a 10-wk period. The individual doses ranged from 1 to 20 mg and a total of 164 mg of antibody was administered. The patient initially responded well to serotherapy; circulating Sézary cells were cleared, and tumor cells in skin and lymph node regressed. A complete remission was not achieved, however, and after 7 wk, tumor cells again began to proliferate despite continued antibody infusions at higher doses. Immunoperoxidase staining of a lymph node biopsy demonstrated that resistant tumor cells were still reactive with L17F12 antibody.

The toxicity of antibody infusion was minimal in this patient. Creatinine clearance decreased transiently following the first antibody infusion but not after any subsequent treatment. Normal circulating granulocytes, monocytes, and B cells were also transiently reduced immediately after each antibody infusion, even though L17F12 did not specifically react with these cells. As expected, normal circulating T cells were persistantly depleted during serotherapy. It is not known whether T cells in lymph nodes were also depleted or whether significant immunosuppression resulted from the reactivity of L17F12 with normal T cells.

In vitro studies identified three factors that may have limited the response to antibody infusion. The first was presence of free circulating antigen. In the patient with T-cell leukemia, serial studies indicated that antigen was released into the circulation following the first antibody infusion and subsequent lysis of leukemic cells. Free antigen was not present prior to serotherapy. The second factor was antigenic modulation. As has been previously described for CALLA, antigenic modulation resulted in the decreased expression of L17F12 antigen following antibody infusion in both patients. The third factor was the host immune response to the murine antibody infusion. In the first patient, this resulted in an IgM antibody response that was directed against the L17F12 antibody. This did not appear to significantly inhibit serotherapy, since it was only detectable on a single day following the first antibody infusion and not after any of the other antibody treatments. No immune response to murine antibody was seen in the patient with T-cell lymphoma.

**B-Cell Chronic Lymphocytic Leukemia**

Dillman et al.\textsuperscript{39} have recently presented the results of monoclonal antibody therapy in 2 patients with chronic lymphocytic leukemia (CLL). The antibody utilized (T101) is specific for a 65,000 dalton protein that is primarily expressed by normal T cells, thymocytes, and malignant T cells\textsuperscript{37} and, therefore, has similar specificity to L17F12 antibody. In addition, B-CLL cells are reactive with T101 antibody. As noted previously for other murine IgG2a antibodies, T101 was able to initiate lysis of appropriate target cells in the presence of rabbit complement but not with human complement. T101 was also unable to initiate antibody-mediated cellular cytotoxicity (ADCC) in vitro.

At the time of serotherapy, both patients had large tumor burdens and, in addition, patient 2 had normal T cells in the peripheral blood that were reactive with T101. In both patients, T101 antibody infusion resulted in rapid clearance of circulating CLL cells, which then returned to pretreatment levels. As expected in patient 2, normal T cells were also transiently affected. Unlike the patients previously discussed, antibody infusions were not well tolerated in these two patients. Patient 1 developed an anaphylactoid reaction after the third antibody infusion, which included urticaria, diarrhea, dyspnea, cough, and hypotension. The second patient developed dyspnea, hypotension, rigger, fever, and elevated hepatic enzymes after the first antibody infusion. Both patients subsequently recovered from these adverse reactions to monoclonal antibody infusion. In vitro studies demonstrated that T101 antibody rapidly bound to both CLL cells and normal T cells in vivo, and it was possible to saturate all antigenic sites. It is not known whether C3 was deposited on these cells after antibody binding, but serum complement levels did not change. The antitumor effect of antibody infusion in this trial was similar to that seen in the other patients that have been reported, but there was no evidence of either circulating blocking factors or antigenic modulation. Despite the anaphylactoid reactions to murine antibody, an antibody response to T101 was not demonstrable.

**IDENTIFICATION OF FACTORS LIMITING THE THERAPEUTIC EFFICACY OF MONOCLONAL ANTIBODY**

Although the generation of monoclonal antibodies reactive with human tumor cells has circumvented many of the problems that have previously limited the effectiveness of serotherapy, it is evident that major limitations still exist. Some of these obstacles are not unexpected, since earlier studies using conventional antisera have encountered similar problems.\textsuperscript{67-69} Nevertheless, the clinical studies that have been reviewed represent very useful experiments that have delineated a series of factors limiting the application of monoclonal antibodies in the treatment of leukemia.
and lymphoma. The identification of these factors is a necessary step in learning how to effectively use these powerful new reagents. Undoubtedly, as more trials with other antibodies are pursued, additional limiting factors will become evident.

Toxicity of Monoclonal Antibody Infusion

In general, the direct toxicity of monoclonal antibody infusion has not been severe, and only in the study reported by Dillman et al.\textsuperscript{39} did toxicity limit further administration of antibody. In this trial, two patients developed anaphylactoid reactions immediately after completion of antibody infusions even though skin tests prior to serotherapy showed no evidence of immediate hypersensitivity. In all other patients, adverse reactions were mild and transient and did not limit further treatment. The major adverse reactions that were noted included transient fevers and decreases in creatinine clearance. The renal toxicity appeared to be secondary to the clearance of circulating immune complexes. Although the total dose and frequency of antibody infusions varied greatly in these studies, one patient received a total dose of 1750 mg without major toxicity.

Serum Blocking Factors

Two of the four clinical studies demonstrated that the presence of circulating antigen effectively blocked monoclonal antibody from binding to leukemia or lymphoma cells in vivo. In the study described by Nadler et al.,\textsuperscript{34} serum blocking factor was present in large quantities prior to serotherapy, and infusion of 1.5 g of antibody could significantly deplete circulating antigen but did not result in significant binding to lymphoma cells in vivo. Miller et al.\textsuperscript{37} were not able to detect blocking factors prior to antibody infusion, but in the patient with T-cell leukemia, circulating antigen transiently appeared after the first antibody infusion. This was presumably a result of lysis of leukemic cells in vivo. Circulating antigen subsequently blocked the effectiveness of the second antibody infusion, but did not block the activity of a later third antibody infusion, which was given when circulating antigen was no longer detectable. Although numbers of circulating tumor cells decreased after this last infusion, there was no subsequent reappearance of circulating antigen. Blocking antigen was not detected during serotherapy in the patient with T-cell lymphoma.

Circulating antigen was not noted in the two other clinical trials,\textsuperscript{35,39} but it appears that at least in some instances, serum blocking factors will significantly limit the usefulness of serotherapy with monoclonal antibodies. The presence of blocking factor does not appear to be predictable and may be determined by characteristics of the tumor-associated antigen and the type of tumor cell that is the specific target of serotherapy.\textsuperscript{70,71} Furthermore, the amount of circulating antigen may correlate with the total number of tumor cells, just as the level of circulating monoclonal immunoglobulin reflects the total number of tumor cells in multiple myeloma. It is, therefore, possible that circulating blocking factors may not be a major obstacle in treating smaller numbers of tumor cells.

Antigenic Modulation

Two of the four studies found that leukemic cells underwent antigenic modulation as a direct consequence of binding of monoclonal antibody to the cell surface in vivo.\textsuperscript{35-38} These findings were confirmed by in vitro studies\textsuperscript{66} demonstrating that modulation resulted in the loss of both antibody and antigen from the cell surface but that leukemic cells were able to reexpress antigen when antibody was no longer present. The loss of antigen is specific and does not appear to involve a loss of other cell surface proteins. Furthermore, antigenic modulation appears to be a specific response of certain cell surface antigens such as CALLA,\textsuperscript{66} surface immunoglobulin,\textsuperscript{72-75}and GP67 (L17F12 antigen) but does not occur with Ia antigens or histocompatibility antigens.

Antigenic modulation has been studied extensively in the case of TL antigen in murine leukemia and other tumor-associated antigens, and it appears that the mechanism of modulation may be different for various antigens.\textsuperscript{76} In 1967, Boyse et al.\textsuperscript{69} demonstrated that antibodies to TL antigen were not protective against a transplantable TL antigen-positive leukemia because these cells rapidly became TL antigen-negative in vivo. This phenomenon was also found to occur in vitro\textsuperscript{77} and subsequent studies have shown that TL modulation results in a rearrangement of TL antigen within the cell membrane in conjunction with TL antibody and other serum factors.\textsuperscript{78,79} This arrangement of TL antigen into microaggregates rendered TL-modulated cells resistant to lysis by additional TL antibody and guinea pig complement but did not result in the removal of TL antigen from the cell membrane.\textsuperscript{76,80} Thus, TL antigen could still be detected on the surface of modulated cells by either indirect immunofluorescence assay, radioligand assay, or cytotoxicity assay using rabbit complement.

These findings are in contrast to the studies of CALLA modulation,\textsuperscript{66} which have shown that CALLA is lost from the cell surface after binding by J5 antibody. The loss of cell surface CALLA is demonstrable by indirect immunofluorescence assays, cell surface radiolabeling studies, and by cytotoxicity assay using rabbit complement. Furthermore,
CALLA appears to be internalized within leukemic cells during modulation.81

**Immune Response to Xenogeneic Protein**

Previous clinical trials using conventional heterologous reagents (usually either horse or rabbit antisera) have noted immediate systemic reactions to the large amount of foreign proteins that were administered. In addition, patients generated an antibody response to these foreign proteins and serum sickness often developed after serotherapy. As noted previously, only 2 of 9 patients who received monoclonal antibody infusion had apparently allergic responses to murine antibody, and it is possible that these patients may have actually responded to other contaminating proteins or toxins contained in the antibody preparation. Since TIO1 antibody reacted with circulating T cells as well as with CALLA cells, it is also possible that the anaphylactoid reactions seen after TIO1 infusions were the result of lysis of these normal cells. No patients developed serum sickness, and only one patient was noted to have an antibody response specific for murine antibody. In this patient, the response was weak and transient and did not appear to limit serotherapy.

The lack of significant antibody response to murine antibody infusion may reflect the relatively small amounts of antibody that were infused, the purity of the antibody preparations, and the route of administration. In addition, each patient had previously received intensive chemotherapy, which may have significantly suppressed their ability to respond to foreign proteins. In future trials, if patients are treated for longer periods of time and prior to receiving extensive immunosuppressive therapy, an immune response to murine monoclonal antibody may become evident.

**Specificity of Monoclonal Antibody**

Another factor limiting the in vivo use of monoclonal antibodies is the specificity of the reagents themselves. In almost all instances, monoclonal antibodies reactive with leukemia or lymphoma cells also react with various normal hematopoietic cells. The reactivity of these reagents with nonhematopoietic tissues has not been extensively evaluated. Ab894 appears to be specific for lymphoma cells, but very few patients have tumor cells that react with this antibody. CALLA is widely expressed in non-T-cell ALL and in various lymphomas but is also expressed by a small number of normal bone marrow cells.85 Effective serotherapy of leukemia would also deplete this normal population of cells. The function of normal CALLA-positive cells is not known, but recent studies have shown that these cells are not committed myeloid precursor cells.85 In addition, patients who have been transplanted with bone marrow from which CALLA-positive cells have been removed in vitro have had reconstitution of all normal myeloid and lymphoid cells, indicating that pluripotent hematopoietic stem cells do not express CALLA.

The reactivity of monoclonal antibody with normal cells becomes even more evident in the clinical studies with T-ALL, T lymphoma, and B-CLL. In both instances, the monoclonal antibodies used not only reacted with leukemic cells but also with normal T cells in peripheral blood, spleen, lymph node, and thymus.85,86 Since these antibodies have no inherent way of distinguishing normal cells from tumor cells, it would be expected that normal T lymphocytes would also be affected by serotherapy and significant toxicity may result from the lysis of these normal cells. Theoretically, the complete in vivo elimination of tumor cells with either L17F12 or TIO1 antibodies would also necessitate the complete elimination of normal T cells. In addition, the presence of a large population of normal antigen-bearing cells would act to inhibit the ability of passively administered antibody to bind to tumor cells and require the infusion of larger amounts of antibody than would otherwise be necessary. It is also likely that significant immunosuppression would result from the depletion of normal T cells.

A major assumption in the clinical application of monoclonal antibodies is that all tumor cells in the population express the surface antigen that is the specific target of serotherapy. The presence of antigen-negative tumor cells prior to antibody infusion or the emergence of antigen-negative cells following treatment would both result in resistance to serotherapy. This potential problem has not been seen in human studies but has been demonstrated in an animal model.85 These experiments showed that in vivo treatment of a murine lymphoma with a monoclonal antibody specific for a cell surface glycolipid resulted in the selection of a population of cells that expressed less of this surface antigen. Unlike antigenic modulation, which was reversible, serotherapy resulted in a permanent phenotypic change in the expression of the glycolipid antigen.

**Inefficiency of Natural Immune Effector Mechanisms**

Perhaps the most significant limitation of serotherapy is the fact that antibodies are not inherently toxic and that binding of antibody to cell membrane does not adversely affect the growth of these cells except in rare instances.85 The cytotoxic effects of antibody are entirely mediated by naturally occurring effector mechanisms such as complement-mediated cytotoxici-
ty, reticuloendothelial clearance, and ADCC. These effector systems are capable of destroying cells only after antibody has bound to the cell surface.

In the clinical setting of leukemia and lymphoma, which has become refractory to standard therapy, the total number of tumor cells is very great (approximately $10^{12}$) and normal immune responses have been blunted by prolonged chemotherapy. In the patient described by Miller et al., the response to infusion of 1 mg antibody was equivalent to the removal of $2 \times 10^{11}$ cells by leukapheresis. This represents two million times the number of cells that could be consistently eradicated in experimental animal systems and yet the clinical response was negligible, since the total tumor cell population recovered within 24 hr. It seems likely that naturally occurring effector systems are not capable of destroying the large bulk of tumor cells that is present in these situations. This problem is compounded when normal cells bind monoclonal antibody in vivo, and, therefore, compete with tumor cells for lysis by the various effector systems.

FUTURE DIRECTIONS

Although the total number of experimental studies is small, much has been learned about the potential utility of monoclonal antibodies in the treatment of leukemia and lymphoma. Specific limiting factors have been identified and future studies will hopefully result in finding ways to circumvent these limitations.

Development of New Monoclonal Antibodies

Some of the problems noted in previous studies may be resolved through the generation of new monoclonal antibodies with different specificity and of different immunoglobulin isotype. For example, antibodies that do not react with large numbers of normal cells may be more effective in eliminating tumor cells. Antigenic modulation appears to be dependent on the type of monoclonal antibody as well as its specificity. We have recently developed an IgM antibody (J13) that is specific for CALLA but does not induce modulation as rapidly as J5 antibody (IgG2a). In addition, all of the reagents used in clinical studies to date have been IgG antibodies, and none of these antibodies has been able to lyse tumor cells in vitro with human complement. Infusion of J5 antibody resulted in the deposition of C3 on leukemic cells, but since in vitro lysis could not be demonstrated, it is unlikely that direct complement lysis occurred in vivo. IgM antibodies are more efficient activators of complement, and this class of antibody may therefore be more effective in vivo. Unfortunately, IgM monoclonal antibodies have not been effective in animal models of serotherapy. Lastly, the problems associated with the administration of foreign proteins can be resolved through the development of human monoclonal antibodies and the technology necessary to make these reagents is now available.

Treatment of Smaller Numbers of Tumor Cells

Experimental animal studies have clearly shown that serotherapy is most effective against small numbers of tumor cells. Conceptually, it is difficult to proceed with “adjuvant” serotherapy when the effectiveness against visible tumor has been minimal. However, for reasons that have been presented, it is unlikely that passive infusion of monoclonal antibody alone will ever be able to eradicate a large bulk of tumor cells. Certainly, surgical excision of a large bulky tumor is ineffective in the face of widespread metastatic disease, but removal of a smaller primary lesion prior to metastasis is often curative. There are of course obvious flaws in this analogy, and serotherapy has not yet been shown to be curative of any human tumor. Nevertheless, it is likely that if serotherapy will be useful in the treatment of leukemia or lymphoma, it will be in the setting of small numbers of tumor cells and in conjunction with other agents that are capable of eradicating bulk disease.

In Vitro Utilization of Monoclonal Antibodies

Many of the obstacles limiting the use of monoclonal antibodies in vivo may be circumvented through the carefully controlled use of these reagents in vitro. In a manipulated environment, blocking factors can be removed, modulation can be inhibited, human complement can be replaced by more active rabbit complement, the total number of tumor cells can be controlled, and repeated treatments can be utilized. All of these manipulations can potentially increase the efficacy of monoclonal antibody treatment.

Studies applying these concepts with conventional heteroantisera have previously been reported, and more recently, a trial using monoclonal antibody in vitro has been initiated within the context of autologous bone marrow transplantation. In this study, patients with relapsed CALLA-positive ALL but without HLA-identical bone marrow donors are transplanted with autologous bone marrow following ablative chemotherapy and total body irradiation. Prior to transplantation, bone marrow is treated in vitro with J5 antibody and rabbit complement to lyse CALLA-positive leukemic cells. Although it is too early to determine whether or not this in vitro treatment has in fact eradicated all leukemic cells, preliminary results are encouraging and all patients have engrafted with treated marrow.

In the future, the in vitro use of monoclonal antibodies may also be extended to the setting of allo-
geneic bone marrow transplantation from histoincompatible donors. Since the donor marrow is normal in this situation, in vitro treatment with monoclonal antibody is not used to rid the marrow of leukemic cells but rather to eliminate T lymphocytes and to prevent fatal graft-versus-host disease (GVHD). Experimental animal studies indicate that this approach is feasible.\textsuperscript{96-97} Nevertheless, it should also be pointed out that GVHD has been shown to have a significant antileukemic effect,\textsuperscript{93,94} and the total elimination of GVHD may actually have an adverse effect on long-term survival of transplanted patients.

**Development of Monoclonal Antibody–Drug Conjugates**

One major finding in two of the clinical studies was that, in the absence of circulating antigen, monoclonal antibody rapidly bound to leukemic cells in bone marrow as well as peripheral blood.\textsuperscript{35,39} Thus, even though all tumor cells were not eliminated, binding of antibody to these cells did occur. Monoclonal antibodies may, therefore, be ideal vehicles with which to deliver cytotoxic agents directly to tumor cells in vivo. In instances where monoclonal antibodies induce antigenic modulation such as with J5 antibody and CALLA, this approach may be even more effective than with other reagents. Since modulation of CALLA has been shown to result in the internalization of this antigen, cytotoxic agents coupled to J5 antibody might also be selectively internalized. Various types of toxic agents may be used and the specific procedures for conjugating some of them to antibodies have already been developed. These include various chemotherapeutic agents,\textsuperscript{95,96} plant toxins such as Ricin-A chain,\textsuperscript{97,99} lipid vesicles loaded with either drugs or toxins,\textsuperscript{100} and radiopharmaceuticals. The use of monoclonal antibodies as carrier molecules for other cytotoxic agents may significantly increase the clinical effectiveness of antibody infusion while at the same time decrease the nonspecific toxicity of these agents.

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