Initial Experience in Treating Human Lymphoma With a Chimeric Univalent Derivative of Monoclonal Anti-idiotype Antibody


Murine monoclonal anti-idiotype antibody was raised against the surface IgM on the neoplastic cells of a patient with widespread follicular lymphoma. For therapy, a chimeric antibody derivative, FabIgG, was constructed by thioether-linking Fab'γ, from the monoclonal antibody, to human normal IgG. FabIgG is univalent and thereby avoids rapid antigenic modulation. Its human IgG component is intended to optimize recruitment of effectors and metabolic survival while minimizing immunogenicity. Four intravenous (IV) infusions of 380 to 580 mg of anti-idiotype FabIgG were given over a period of 11 weeks. There was no significant toxicity. On each occasion, the antibody disappeared from the plasma with a half-life (t1/2) of <24 hours. The brief survival was evidently due to uptake by tumor, as infused control FabgG, containing Fab'γ from an irrelevant antibody, yielded a plasma t1/2 of >10 days. With each therapeutic infusion, there was a fall in the number of circulating neoplastic cells over a 24-hour period. The numbers were largely replenished over the next week, but a net fall became discernible over the entire period of treatment. Four days after each infusion, nodal masses were swollen and tender, subsiding over ~8 days. At the end of the treatments, the blood lymphocyte count and nodal and splenic swellings continued to subside, so that by 6 weeks a partial remission with removal of >50% of tumor was judged to have occurred. We did not detect any qualitative change in surface idiotype nor any antibody response to the infused Ig.

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THE TREATMENT OF lymphoid malignancies with antibody has to date yielded quite modest results, with most patients showing only partial or transient remissions.1 Included in this experience is the use of anti-idiotype antibody (anti-Id) directed against variable regions of tumor surface immunoglobulin (Ig).2-7 The target represents a uniquely well-characterized differentiation antigen, of specificity so narrow that at present an individual anti-Id must be raised for each tumor to be attacked.8 Monoclonal technology has improved therapeutic prospects by making anti-Id available as a homogeneous reagent in indefinitely large quantities but, despite initial promise, its inherent antitumor effect is unlikely to be better than that of earlier polyclonal reagents when given in unmodified form.

Factors limiting the therapeutic efficacy of anti-Id are by no means fully defined.1,8 They probably include sparse surface Ig, an appreciable secretion of the tumor Ig, idiotypic heterogeneity within a tumor, antigenic modulation, the failure of antibody to recruit appropriate effectors (the roles of complement and the various cellular effectors are poorly understood), and induction of an anti-antibody immune response. Some of these factors can be taken into account when cases suitable for therapy are selected, and some can be by designing suitable derivatives of antibody. For example, experience has emphasized the difficulties involved in treating with anti-Id those lymphomas secreting appreciable idiotypic Ig into extracellular fluid;7 we showed in animal lymphoma that certain univalent antibody derivatives, which avoid antigenic modulation, are therapeutically superior to the bivalent parent antibodies.9,10

In this article, we present initial data on the therapeutic use in humans of an antibody derivative designed to avoid or minimize several of the above problems. The derivative is the chimeric univalent antibody, FabIgG (Fig 1). It consists of Fab'γ from mouse monoclonal anti-Id, linked by tandem thioether bonds to human normal IgG. Preparation and preliminary characterization of FabIgG have been described.11 Being univalent, it avoids rapid antigenic modulation.12,13 The possession of a human Fcy region, within the IgG moiety, is designed to optimize recruitment of effectors, prolong the metabolic half-life (t1/2),14 and minimize immunogenicity.1 The first patient to be treated has undergone a partial remission of lymphoma and yielded data supporting the above contentions.

MATERIALS AND METHODS

Patient. B.R., a white woman, was seen in October 1983 at the age of 51 for a lump in the left axilla which on excision biopsy proved to be non-Hodgkin's lymphoma (centroblastic/centrocytic, nodular). No other lymphadenopathy was detected, but her spleen was palpable on inspiration. The blood showed hemoglobin (Hb) 13.3 g/dL, WBC count was 14.2 × 109/L, and platelet level was 225 × 109/L. The differential count was neutrophils 49%, lymphocytes 47%, and monocytes 4%; among the lymphocytes, small cleaved cells and centroblasts were prominent. Marrow aspirate revealed increased numbers of small cleaved lymphocytes, and paratrabecular infiltrates of lymphoma were seen on a trephine sample. Serum Ig levels were normal. Urinary Ig comprised κ 1.3 mg and λ 1.9 mg/24 hours; no clear evidence of a monoclonal component was seen on immunofixation of an isoelectric focusing pattern from concentrated urine. A lymphangiogram revealed enlarged nodes with abnormal patterns in paraaortic, paraaortic, and inguinal regions. Studies of lymphocytic surface markers are summarized in Table 1. Blood lymphocytes stimulated with phorbol ester and pokeweed mitogen (PWM) showed Trisomy-12 on karyotyping. Leukapheresis in November 1983 yielded ~1010 lymphocytes after separation over

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Lymphoprep (Nyegaard, Oslo). These were used for studies in vitro and to derive tumor Ig for immunization.

The patient remained asymptomatic and was observed without treatment. In January 1985, examination revealed a splenic edge 4 cm below the costal margin and enlarged mobile lymph nodes as follows: left axilla 2 × 2 cm; left submandibular, two adjacent together 4.5 × 2 cm; left inguinal, two groups 4 × 1 and 2 × 2 cm; and right inguinal 1 × 1 cm. The leukemic overspill had not increased commensurately: WBC count was 11.2 × 10^9/L, lymphocytes 69%.

We decided to see whether the patient's tumor burden could usefully be reduced by antibody before resorting to conventional chemotherapy. Informed consent was obtained from the patient, and ethical clearance was granted by the East Dorset District Health Authority Research and Ethical Committee.

**Marker studies.** Blood lymphocytes and dispersed cells from the excised lymph node were prepared as described previously. Two thioether bonds link the two components through a phenylene ring. Attachment to Fab'γ is probably at a hinge 1/2-cystine as shown. Attachment to Ig has a high probability of being at one of the interchain 1/2-cystines, but is not known to show any preference among them. Residual interchain disulfide bonds are indicated by narrow lines.

**Table 1. Lymphocytic Surface Markers**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Marker (Dispersed Cells)</th>
<th>Blood Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep RBCs</td>
<td>CD2</td>
<td>20</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>CD3</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>CD5</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CD10</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>HLA-DR (β)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CD22</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>μ chain</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>δ chain</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>κ chain</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>λ chain</td>
<td>74</td>
</tr>
<tr>
<td>Polyclonal purified anti- Ig</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>71</td>
<td></td>
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<tr>
<td></td>
<td>85</td>
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</table>

ND, not done.
revealed strong cross-inhibition. “Anti-Id IgG” henceforth refers to this pool of two antibodies.

F(ab’2)2 was prepared from the pooled IgG1 preparation by digestion with papain under standard conditions.13 IgG1 20 mg/mL, papain 0.2 mg/mL in 0.1 mol/L of acetate buffer of pH 4.2, incubated at 37 °C for 18 hours, followed by chromatography on Sephacryl S300 (Pharmacia, Uppsala, Sweden) equilibrated in 0.1 mol/L of Tris HCl, pH 8.0.

Preparation of FabIgG. The preparation of FabIgG has been described in detail.14 F(ab’2)2 from antibody is reduced with 2-mercaptoethanol under the minimal conditions, giving a high yield of monomeric Fab’. Next, the liberated SH groups are allowed to react with a surplus of o-phenylenediamineleimide to give Fab’y bearing a small number of active maleimide groups attached predominantly to its hinge region. The Fab’y then reacts with human normal IgG that has previously been reduced with a limiting amount of dithiothreitol to give an average of <3.0 SH groups per molecule. The two reactants are mixed in a molar ratio of Fab’y:IgG = 1:2 and incubated at 5 °C for 24 hours. The FabIgG is then separated from larger products and from residual reactants by reducing chromatography through Sephacryl S300 (Pharmacia) equilibrated with PBS. Typically, ~600 mg of FabIgG is collected from the column system in ~1,100 mL. Some surplus normal IgG contaminates the tail of the FabIgG zone, but this is regarded as of no consequence for therapeutic purposes. The yield of Fab’y in the chimeric antibody was usually ~40% of that used in the synthesis.

Infusion of antibodies. The procedure for infusion of antibodies was essentially the same for both IgG and FabIgG. Any aggregated protein was removed from the preparations by passage through Sephacryl S300 and was equilibrated with sterile, pyrogen-free, PBS. For FabIgG, this corresponded with the purification procedure described previously. From the S300, the final antibody-containing effluent was led through a polymyxin column to remove any pyrogens, and then through a 0.22-μm filter (Millipore, Harrow, England) directly into a sterile IV giving set. Aliquots from the giving set were tested for pyrogens by the limulus amebocyte method (Pyrogen test, Mallinkrodt, Round Spinney, Northants, England) and always proved negative.

Twenty-four hours before the infusion was started, allopurinol 300 mg daily was started. At this time also, a serum sample was examined by micro-Ouchterlony technique for precipitins to mouse Ig, the method being capable of detecting antibody at <10 μg/mL. One hour before the infusion, a cutaneous prick test was performed to test for immediate hypersensitivity to the antibody.

Infusions, consisting of 200 mg of anti-Id IgG1 or 380 to 580 mg of anti-Id FabIgG, were given in a volume of 600 to 1,200 mL over 6 hours. The infusions of FabIgG were accompanied by up to 300 mg of human normal IgG, representing incompletely separated surplus reactant. A close watch was kept on temperature, pulse, and respiration.

Microassays. Our general procedure for ELISA has been described.15 For measurement of either FabIgG or mouse IgG in the patient’s serum, the plate was coated with affinity-purified rabbit anti-mouse Ig at 5 μg/mL; this antibody had previously been absorbed with human Ig. The standard antigen was FabIgG or mouse IgG, corresponding to the unknown, used at starting concentrations of 200 and 20 ng/mL, respectively. Detection of bound antigen used the same rabbit anti-mouse IgG, conjugated to horseradish peroxidase and used at a dilution of 1:500.

To assay the patient’s serum for the presence of idiotype-positive IgM, the ELISA plate was coated with monoclonal anti-Id using a solution at 5 μg/mL; the standard antigen was the supernatant of the IgM-secreting rescue hybridoma, and the detecting antibody was affinity-purified goat anti-human μ chain conjugated to horseradish peroxidase (1:1000) used with normal mouse serum (1:500).

Assessment of antibody in vitro. Tests for antibody-mediated complement lysis and for antibody-dependent cellular cytotoxicity (ADCC) were carried out by standard methods as previously described.16 Distribution of surface antigen–antibody complexes was visualized by indirect immunofluorescence,17 with flow cytofluorimetry carried out on the Becton Dickinson FACS III system.

RESULTS

Studies on tumor cells in vitro. Markers found on nodal and blood lymphocytes are summarized in Table 1. The surface Ig (IgMA, with IgD just detectable on a minority of cells) was at a low level on all cells, being about equal to the average density observed in cases of chronic lymphocytic leukemia (CLL). About half the cells showed detectable class II histocompatibility antigen, whereas all stained for CD5, a pan-T marker that also appears on most cases of CLL and on some B cell lymphomas; in our experience, these findings are unexceptional for this type of lymphoma.

A sample of blood lymphocytes prepared in February 1984 appeared from marker studies to consist of at least 80% tumor cells. When the cells were cultured for 6.5 hours in vitro, they secreted Ig as follows21: X chain 1,500 and λ chain 6,400 dimeric molecules per cell per hour and IgM 130 monomeric molecules per cell per hour. This represented minimal IgM secretion by the tumor, and led to the expectation, confirmed later, that only a small barrier of idiotypic Ig existed in the patient’s plasma.

As we observed earlier7 with tumor cells having sparse surface Ig, no anti-Ig antibody tried would invoke complement lysis, using either human AB or rabbit serum as a source of complement. The antibodies tried included the monoclonal anti-Id, the FabIgG prepared from it, sheep polyclonal anti-Fdμ, and sheep polyclonal anti-λ. Neither would any of these antibodies invoke ADCC in the presence of human normal blood lymphocytes at an effector to target ratio of 50.

Fig 2. Modulation of blood lymphocytes in vitro by IgG1 and FabIgG anti-idiotype antibody (anti-Id) shown by cytofluorimetry of indirect immunofluorescence. X-axis, fluorescence (linear scale set at half-maximum gain); y-axis, cell number. Indicator antibodies: for IgG1, fluoresceinated rabbit anti-mouse IgG; for FabIgG, fluoresceinated rabbit anti-human Fcy. Control (0°C): cells (1.8 × 10^7/mL) and antibody (IgG1 100 μg/mL, FabIgG 250 μg/mL) were incubated at 0°C for 30 min; indicator antibody was then added, and the suspension was maintained chilled during examination. Test (37°C): cells and antibody were incubated at 37°C for 30 min; they were then chilled, and indicator antibody was added. FabIgG at 37°C induced only a minimal change in profile. Peaks nearest the y-axis are antigen negative.
Modulation, in the sense of clearing of antigen–antibody complexes from the tumor cell surfaces, occurred sluggishly in vitro in the presence of monoclonal anti-Id at 37 °C: after 30 minutes, possibly 50% of the integrated fluorescence seen on flow cytofluorimetry had disappeared. Modulation by FablgG over the same period was slight or undetectable (Fig 2), whereas modulation by its Fab'y component alone was never observed.

Infusion of anti-Id IgG1. An infusion of 200 mg over 6 hours was tolerated without any symptoms. Changes seen in the blood lymphocyte count are shown in Table 2. The lowest count recorded occurred in midinfusion; from this point, the count rose steadily to its former level over 1 week. The tumor masses did not change. This is very similar to what we have seen previously with infusions of polyclonal anti-Id3,5 and to what has been recorded for therapy with a variety of monoclonal antibodies.1

Infusions of FablgG. The clinical effects of each of four infusions were very similar. No symptoms were noted during the infusions. Beginning 6 hours after completion and continuing for 24 hours, the patient suffered nasal congestion, edema of the eyelids, mild pyrexia (37.5 °C), and mild abdominal pain. Four days after completion of infusion, lymph nodes in all areas were swollen, reaching about twice their previous size, remaining enlarged for 5 to 8 days, and then subsiding. There were no corresponding changes in splenic size.

Lymphocyte counts at midinfusion showed falls similar to that observed with IgG1 antibody (Table 2). The postinfusion nadir did not occur immediately after the infusion, however, but ~1 day later. From this point, the count again rose slowly, with the troughs more prolonged than after the IgG1 infusion.

A rise in C3c concentration in serum was evidence of complement activation. Thus, 7 hours after the second FablgG infusion was started, crossed immunoelectrophoresis gave a strong peak for C3c. This was maximal at 24 hours and subsiding at 48 and 72 hours.

Following the third FablgG infusion, with the associated nodal swelling and subsidence, a marked net diminution in the size of palpable tumor was obvious. Physical examination showed that the cervical masses had shrunk to ≤50% of their initial volume and that the splenic edge had retreated to the costal margin. In the 11 weeks following the fourth FablgG infusion, the tumor masses either remained stationary or subsided further, whereas the blood lymphocyte count diminished to 3.1 × 10⁹/L, ~40% of the pretreatment level (Fig 3).

In the ensuing 4 months, the patient remained well, with tumor masses unchanged and hemoglobin and granulocyte levels well maintained. The lymphocyte count, remarkably, dropped even more, to 2.4 × 10⁹/L. Of these cells, >80% were idiotype-positive and only 7% were scored as T cells. On the tumor cells, the surface Ig appeared to be at about its original density, and reaction with the monoclonal anti-Id was undiminished. At no time during or after antibody treatment could antibody to mouse Ig be detected in the patient’s plasma.

Plasma idiotype and antibody levels. Throughout the period of treatment, the plasma idiotypic IgM was at 0.9 to 1.4 μg/mL, except during the immediate postinfusion periods when, with free antibody in the plasma, the concentration fell to <0.3 μg/mL. These levels are much lower than those we have found in most B cell neoplasms.8

After all antibody infusions, both IgG1 and FablgG, the antibody (assayed as mouse Fab'y) disappeared from the plasma with a t½ of <24 hours. In the later FablgG infusions, this was regarded with disappointment, as calculations had suggested that the antibody should have been sufficient to swamp both the extracellular and the cell surface idiotypic IgM. We believed it was important to confirm that the FablgG was being consumed rapidly because of its antibody activity and not because of some unexpected metabolic fate such as scavenging by macrophages. The patient therefore consented to a control infusion

<table>
<thead>
<tr>
<th>Antibody Preparation</th>
<th>Day of Infusion</th>
<th>Counts (10⁹/L) at Times Related to Starting Infusions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>−1 h</td>
</tr>
<tr>
<td>IgG1</td>
<td>200 mg</td>
<td>1</td>
</tr>
<tr>
<td>FablgG</td>
<td>480 mg</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>380 mg</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>450 mg</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>580 mg</td>
<td>92</td>
</tr>
</tbody>
</table>

*At midpoint of infusion.
of FabIgG in which the Fab'γ moiety had anti-Id activity against guinea pig L2C cells and no activity against either her tumor cells or normal human lymphocytes; the IgG moiety was again normal human. Two weeks after the fourth therapeutic lot of FabIgG, the patient received a 6-hour infusion of 150 mg of the control material. There were no symptoms and no significant changes in lymphocyte counts. Plasma levels of mouse Fab'γ estimated for 1 week after the infusion revealed a t\(^1/2\) > 10 days (Fig 4), comparable with the value of ~20 days expected for human normal IgG.\(^{23}\)

Qualitative studies of tumor cells. Reports on blood films from the hematology laboratory always indicated an increase in “smear” cells among the lymphocyte populations in immediate postinfusion periods: for example, these cells usually represented 10% to 15% of lymphocytes before an infusion but were reported at 50% 24 hours after the IgGl antibody and at 43% 24 hours after the first lot of FabIgG antibody.

Observations of bound antibody and of modulation of surface antigen-antibody complexes were rendered difficult by the sparseness of surface Ig. Immediately after the IgG1 infusion immunofluorescence detected a diminution in the surface Ig; this was attributed to modulation. More surprisingly, flow cytofluorimetry revealed similar decreases in surface Ig after infusions of FabIgG. In Fig 5, the distributions of surface Ig on blood lymphocytes are shown at times related to the first FabIgG infusion. At 7 hours, 1 hour after the end of the infusion, all three anti-Ig antibodies used revealed diminished reactivity. At 24 hours, the profiles were essentially unchanged. At 3 days, the reactivities were still not completely restored, especially with anti-Id. Very similar appearances occurred after the ensuing three FabIgG infusions.

The diminution in detectable surface Ig may have been due to blocking of indicator antibodies by therapeutic antibody, to modulation, or to preferential removal by therapeutic antibody of cells with higher than average densities of surface Ig. We believe that modulation is probably the major cause, although patching and internalization could not be judged confidently by fluorescence microscopy. Residual therapeutic antibody could not have had a significant blocking role, as none could be detected on the cells by fluorescein-ated anti-human Fcγ. Finally, the diminution in lymphocyte count at 7 hours, even if highly selective, does not seem sufficient in itself to have yielded so pronounced a change in surface Ig profiles. At no stage related to any of the infusions was the complement component C3 detected on blood lymphocytes by immunofluorescence.

Needle aspirates of cervical tumor masses were taken 14 days after the first FabIgG infusion as the masses were subsiding, and 2 days after the third FabIgG infusion before any infusion-related changes in size occurred. In the first aspirate, cytospin preparations revealed 74% tumor cells (λ-bearing) and 23% T cells (CD3-bearing). In the second aspirate, these figures were 69% and 21%, respectively; the tumor cells were predominantly large and atypical, many with one to three nucleoli. In neither preparation could the infused antibody be detected on cell surfaces.

DISCUSSION

No conclusions about the therapeutic efficacy of FabIgG are drawn from this case except that the derivative is at least capable under certain conditions of reducing tumor load. Non-Hodgkin’s lymphoma is notorious for waxing and waning without obvious cause or following an apparently irrelevant event such as intercurrent infection.\(^{24,25}\) In the present case, there is no obvious reason for the reduced amount of tumor having apparently stabilized after termination of the
infusions, with indeed an actual further reduction in the number of circulating neoplastic cells. Clearly, one must bear in mind the possibility that antibody treatment of lymphoma may not only be cytotoxic, but may also trigger poorly understood immunoregulatory pathways.26

Because of overall disappointing results in antibody therapy in lymphoma,1,5-7 some modification of the approach appears indicated. Apart from the mere addition of antibody to existing drug schedules, two main lines of development are being considered. One is the use of antibody as a vector to deliver a more lethal agent (radioisotope, tumoricidal drug, plant or bacterial toxin) to the cell. The other, adopted here, attempts to make better use of the cytotoxic effector mechanism normally recruited by antibody. The best known of these mechanisms are complement, phagocytes, and K cells. Unfortunately, we have no clear indications of their relative importance in dealing with antibody-coated lymphoma cells.8 We can assert, however, that a major advantage of relying on any of these mechanisms is that they contain an inbuilt safety factor in addition to the specificity of antibody: to activate any of the effectors, antibody must form a sufficient array on the cell surface, oriented with its Fc zones outward. Weak cross-reactions of the antibody with normal cells or the uptake of antigen-antibody complexes by bystander cells are unlikely to be harmful.

Univalent derivatives of antibody which retain intact Fc zones are superior to their bivalent parent antibodies in invoking killing of mammalian cell targets by natural effector mechanisms.9,11,27 Their superiority derives from their avoidance of rapid antigenic modulation, which can afford significant protection to a cell confronted simultaneously by bivalent antibody and an effector such as complement.13,28 The chimeric univalent antibodies FabFc and FabIgG (Fig 1) are developments of the univalent concept, presenting additional advantages from the possession of a human Fcγ zone. Such derivatives clearly are potentially varied and—especially if one includes molecular genetic and hybridoma technologies—might be constructed in a large variety of ways.

With the target cells of this study, neither FabIgG nor the parent IgG1 antibody appeared to activate complement or ADCC in vitro. We suspect that the failure of FabIgG is due to the sparseness of surface antigen.5 Against different target cells, chimeric univalent antibodies, including FabIgG, have clearly demonstrated their abilities to activate both the effector systems.11,29,30

Despite their lack of cytotoxic activity in vitro, both IgG1 and FabIgG antibodies reduced the numbers of circulating neoplastic cells after infusion. If each drop in lymphocyte count reflects cell death, the effector mechanisms must be more useful in vivo than is suggested by performance in vitro. But could the drop be due more to temporary sequestration than to death of the cells? Attempts to answer this have been made in earlier studies31,32 by infusing antibody after indium-labeling of the blood lymphocytes. The results suggested cell death as the main mechanism because labeled cells disappearing on antibody infusion did not subsequently reappear in the blood in quantity. In the present work, the simple observation of a marked increase in smear cells suggests a range of damage compatible with extensive cell death.

We are not aware of any previous report of tumor becoming swollen and tender in response to antibody therapy in the manner shown by the nodal masses in our patient. Because it occurred only with FabIgG infusions, and because the FabIgG but not the IgG1 antibody activates complement, it is tempting to regard this inflammation as complement-induced. The delay in onset for several days after each infusion is then difficult to explain, however. Examination of nodal aspirates did not help: in particular, granulocytes evidently were not recruited by complement peptides. Unfortunately, our patient was unwilling to undergo an excision biopsy that might have revealed more useful information. Garcia and colleagues33 recently described the lymphoid histology in ten patients treated with monoclonal anti-Id, with evidence that clinical remission is more likely the greater the number of infiltrating T cells and the greater the incidence of CD4 (helper), CD25 (TAC), and Leu-7 (NK) positivity among them.

The mild symptoms suffered by the patient ~6 hours after each FabIgG infusion were possibly due to complement anaphylatoxins and/or to interleukin-1 from stimulated macrophages.

The modulation of surface Ig which appeared to be induced by FabIgG in vivo exceeded anything inducible by the same derivative in vitro. Evidence shows for the mouse Ia system that antibodies that do not modulate in vitro can do so in vivo.5 Monocytes in the capacity of bystander cells have clearly promoted or even induced modulation of antibody on target cells,35 raising the possibility that enhanced modulation in vivo in the present case was due to interaction of the bound FabIgG with Fcγ-receptors on various cells.

Although the modulation by bivalent and univalent antibodies in vivo appeared similar, the time course cannot be followed with the same precision as is possible in vitro. The speed with which bivalent anti-Ig can induce modulation in vitro is sufficient to provide significant protection to a cell exposed simultaneously to antibody and complement.13 Neither we nor others observed such rapid modulation by univalent antibody.12,13 It therefore remains entirely possible that the modulation induced by univalent antibody in vivo is slower, and hence less protective, than that induced by bivalent antibody.

At the time of the fourth FabIgG infusion, the total idiotype IgM harbored by the patient was estimated crudely at <50 mg based on a plasma level of 1 μg/mL, an extravascular fluid load equal to the intravascular fluid load, a tumor burden <2 × 1012 cells, 20,000 molecules of monomer per cell surface, and an intracellular pool of the same size. The rate at which the infused antibody disappeared, in contrast to nonantibody FabIgG (Fig 4) indicates that our calculation was a gross underestimate. (Another explanation for the much briefer survival of the antibody FabIgG is that the patient herself was mounting an anti-Id response against the antibody. No increase in blocking activity was present in her plasma to support this suggestion.) Possibly, the large spleen contained unsuspected quantities of idiotypic Ig, although findings by Meeker and
co-workers on two similar patients subjected to splenectomy do not support this notion. It can be expected that saturation of antigenic sites would leave surplus antibody FabIgG with 1/2 as much of Fab than of F(ab')2. With such a metabolic survival, the FabIgG would represent quite an economic means of using antibody to provide a prolonged attack on the tumor.

No anti-mouse Ig response has been observed in this patient. In animals, FabIgG containing homologous IgG but xenogeneic Fab elicits much lower antibody response than does the corresponding xenogeneic IgG.11 Therefore, there are grounds for hoping that avoidance of xenogeneic Fab will reduce the problem of anti-Ig responses by the patient following therapy with monoclonal antibody. The idiotypic and mouse F(ab')2 determinants on FabIgG remain potential immunological targets. We shall continue to observe this patient closely. Our present intention is to reinstitute antibody treatment when the neoplasm appears to be expanding again.

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