Preexisting Human Anti-Murine Immunoglobulin Reactivity Due to Polyclonal Rheumatoid Factors

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ABSTRACT

We report that sera from healthy controls, patients with ovarian or lung cancer, and patients with rheumatoid arthritis all contain IgM polyclonal rheumatoid factors which recognize antigenic determinants on murine and to a greater extent human immunoglobulin IgG. The major part of this reactivity is directed against conserved, shared antigenic determinants present on both human and murine IgG. Such antigenicity resides in the protein and not the carbohydrate moiety of IgG, since deglycosylation of the target murine monoclonal antibody did not result in any loss of antibody binding.

Studies comparing the binding of polyclonal and monoclonal rheumatoid factors (from patients with rheumatoid arthritis and mixed essential cryoglobulinaemia, respectively) to murine and human IgG show that the antigenic determinants recognized by polyclonal rheumatoid factors are present on both whereas the antigenic determinant recognized by the monoclonal rheumatoid factors is present only on human IgG. Furthermore, patients with rheumatoid arthritis display an elevated human IgM anti-murine immunoglobulin response similar to that seen in cancer patients who have received murine monoclonal antibody therapy.

We therefore conclude that, where possible, F(ab')2 fragments of murine monoclonal antibodies should be used for in vivo tumor localization studies to avoid possible immune complex formation, and that patients with rheumatoid arthritis should be considered to be possibly at higher risk of developing immune complex disease, were these rheumatoid factors to bind to the administered murine antibodies in vivo.

INTRODUCTION

The clinical use of murine monoclonal antibodies is increasing, for example in areas such as the treatment of renal allograft rejection (1–5) and in tumor diagnosis and therapy (6–22). However, administration of murine monoclonal antibodies to patients results in the development of a human anti-murine immunoglobulin response (23–29). The development of such a response is understandable; what is less clear is the origin of "preexisting" anti-murine immunoglobulin antibodies which have been found in sera from healthy individuals and patients with various diseases (30–33). We have previously shown that such preexisting antibodies are directed against antigenic determinants on the constant, Fc, portion of the immunoglobulin molecule and that a significant proportion of these antibodies are of the IgM immunoglobulin class (30).

Other studies have reported the presence of similar antibodies in both patients' and healthy individuals' sera that have been shown to interfere with assays for thyrotropin (34), creatinine kinase—MB isoenzymes (32), and ferritin (35). Moreover, some authors have proposed that false hyperthyrotropenaemia may be induced by heterophilic antibodies (36) and that heterophilic antibodies may be responsible for false positive serological tests in heart transplant recipients (37).

Finally, it is well known that rheumatoid factors, found in the serum of patients with rheumatoid arthritis, are predominantly IgM autoantibodies with specificity for self IgG (38). The autoantigenic determinants involved have been located to the , constant domain of this IgG (39, 40).

We report here that the preexisting anti-murine immunoglobulin response is due to the presence of polyclonal rheumatoid factors with specificity for protein antigenic determinants that are present on the Fc region of both human and murine immunoglobulin. Furthermore, rheumatoid arthritis patients with elevated serum rheumatoid factors display an elevated anti-murine immunoglobulin response.

MATERIALS AND METHODS

Sera. Serum samples were obtained from: (a) healthy controls (blood donors); (b) patients with ovarian and lung cancer prior to, and postadministration of radiolabeled murine monoclonal antibodies for tumor localization and/or therapy; and (c) from patients with rheumatoid arthritis. IgM polyclonal and monoclonal rheumatoid factors were purified by affinity chromatography from the serum of patients with rheumatoid arthritis and mixed essential, or type II, cryoglobulinaemia, respectively. All were aliquoted and stored at −20°C.

Monoclonal Antibodies. Murine monoclonal antibodies (HMFG1 and AU1) used as antigen in the enzyme-linked immunosorbant assay were purified from tissue culture supernatants by protein A chromatography. Purity was assessed by both sodium dodecyl sulphate-polyacrylamide gel electrophoresis and isoelectric focusing. Antibodies were diluted to 1 mg/ml, aliquoted, and stored at 4°C until used.

HMFG1 is a mouse IgG1 antibody that binds to a large mucin-like molecule normally produced by the lactating breast, but also expressed by the majority (90% of ovarian, lung, breast, and other carcinomas (41)). AU1 is a mouse IgG1 antibody which detects an antigen expressed by a wide range of adenocarcinomas, including approximately 75% of carcinomas of the ovary and lung (42). Both antibodies (radio-labeled) are used experimentally at the Hammersmith Hospital for tumor imaging and therapy.

Purified Rheumatoid Factors. Four monoclonal rheumatoid factors were isolated from patients with mixed essential cryoglobulinaemia; all were IgM κ protein. They were initially separated from serum as cryoglobulins and then fractionated on sepharose 6B in acetate buffer, pH 4.0, as previously described (43). The polyclonal IgM rheumatoid factors were obtained from patients with rheumatoid arthritis, and were separated by affinity chromatography (anti-human λ).

Deglycosylation of Monoclonal Antibodies. Deglycosylation of murine IgG1 monoclonal antibodies AU1 and HMFG1 was carried out by the method of Thorpe et al. (44). Briefly, this was achieved by concentrating the murine monoclonal immunoglobulin to 2.5 mg/ml followed by dialysis against 0.2 M sodium acetate buffer, pH 3.5, for 16 h. The dialyzed antibodies were then mixed with an equal volume of a solution containing 80 mM sodium cyanoborohydride and 40 mM metaperiodate in acetate buffer, pH 3.5. The mixture was left on ice at 4°C for 20 min. in a fume cupboard and the reaction was stopped by adding 100 µl of 20% glycerol for every 2.8 ml of total sample reaction mixture. This was then left overnight at 4°C, after which it was dialyzed against 3 x 5 liters of 0.05 M ammonium hydrogen carbonate and finally 1 x 5 liters of phosphate buffered saline, pH 7.

The mixture of sodium metaperiodate and sodium cyanoborohydrate at pH 3.5 results in the oxidative cleavage of the carbohydrates and reduction of the aldehyde groups thus formed to primary alcohols. Conducting the procedure at pH 3.5 minimizes both the possibility of...
A comparison of the binding of preexisting IgM antibodies to murine IgG (A) and human IgG (B), using serum from a healthy individual and IgG-coated ELISA plates.

Fig. 2. A comparison of the binding of purified IgM polyclonal rheumatoid factor (PRF, from patient with rheumatoid arthritis) to murine IgG (A) and human IgG (B), using an ELISA system.

Fig. 3. A comparison of the binding of purified monoclonal rheumatoid factor (MRF, from patient with mixed essential cryoglobulinaemia) to murine IgG (A) and human IgG (B), using an ELISA system.

in this assay was purified from a patient with active rheumatoid disease. This patient's sera was found to contain IgM rheumatoid factor at a level of 13 g/liter.

The assay was carried out comparing the response to both murine and human IgG under identical experimental conditions by coating wells in rows 1 to 6 of a 96-well microtiter plate with 500 ng/well of murine IgG and wells in rows 7 to 12 with 500 ng/well of purified human IgG.

As illustrated in Fig. 2 the polyclonal rheumatoid factor

RESULTS

Preexisting Human IgM Anti-Murine and Anti-Human IgG Response. In order to compare the preexisting IgM anti-murine and anti-human IgG response we coated a 96-well microtiter plate with 500 ng/well of murine IgG in rows 1 to 6 and the wells in rows 7 to 12 with 500 ng/well of purified human IgG. This enabled us to compare the response to both IgGs under identical experimental conditions.

Preexisting human IgM antibodies to both murine and human IgG were detected in sera from all healthy controls and patients, except those with mixed essential cryoglobulinaemia where only an anti-human IgG response was detectable (Table 1). All sera that showed binding to both human and murine immunoglobulin displayed a higher degree of reactivity to the human IgG (Fig. 1).

Binding of Purified Human Polyclonal Rheumatoid Factor to Murine and Human IgG. The polyclonal rheumatoid factor used in this assay was purified from a patient with active rheumatoid disease. This patient's sera was found to contain IgM rheumatoid factor at a level of 13 g/liter.

The assay was carried out comparing the response to both murine and human IgG under identical experimental conditions by coating wells in rows 1 to 6 of a 96-well microtiter plate with 500 ng/well of murine IgG and wells in rows 7 to 12 with 500 ng/well of purified human IgG.

As illustrated in Fig. 2 the polyclonal rheumatoid factor

Table 1 Human IgM anti-IgG Response

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of patients studied</th>
<th>Binding to murine IgG</th>
<th>Binding to human IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD</td>
<td>24</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OC</td>
<td>13</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MEC</td>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RA</td>
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</tr>
<tr>
<td>LC</td>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PRF</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* BD, blood donor; OC, ovarian cancer; MEC, mixed essential cryoglobulinaemia; RA, rheumatoid arthritis; LC, lung cancer; PRF, purified polyclonal rheumatoid factor.

Schiff's base formation between aldehyde groups and amino groups in the protein and the possibility of nonspecific oxidation of amino acids.

The stability of the deglycosylated monoclonal antibodies was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing. The immunoreactivity was determined by comparison with the intact antibody in an ELISA.2

Human IgM Anti-Mouse IgG Immunoglobulin Response. Monoclonal antibodies were diluted to 5 μg/ml in bicarbonate buffer (pH 9.6, coating buffer) and used to coat 96-well microtiter plates (Sterilin, Middlesex, England), 100 μl/well (500 ng/well). The plates were incubated at 37°C overnight then washed in phosphate buffered saline, pH 7.4, containing 0.05% Tween-20.

Serial dilutions of each patient's serum together with control sera were made and the plates incubated for 2 h at 37°C, washed three times in PBS + 0.05% Tween (3 × 2-min washes), then incubated for 1 h at 37°C with 100 μl of a 1:500 dilution of a peroxidase-conjugated rabbit anti-human μ-chain-specific reagent (Dako, Denmark).

Plates were then washed three times and incubated at room temperature with 100 μl of 2,2'-azino-di-(3-ethylbenzthiazoline sulphate); (Amersham International, U.K.), and the absorbance was determined in a Titertek Multiscan plate reader (Flow Irvine, Scotland) at 405 nm.

Anti-Human IgG Immunoglobulin Response. The anti-human IgG assay was carried out in an identical fashion to that described above for the anti-mouse IgG immunoglobulin response except that the plates were coated with 500 ng/well of purified human IgG (Sigma, U.K.).

Antidiotypic Antibodies to a Purified Monoclonal Rheumatoid Factor. Purified human monoclonal rheumatoid factor from a patient with mixed essential cryoglobulinaemia was previously used as antigen to raise an antidiotypic mouse monoclonal antibody C8E3 which is an IgGl that binds to a highly restricted idiotope found only on the human monoclonal rheumatoid factor against which it was raised (43).

2 The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline.
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Fig. 4. Monoclonal antiidiotypic antibody C8E3 inhibits the binding of monoclonal rheumatoid factor to its target antigen, human IgG (ELISA system).

Fig. 5. Comparison of the binding of IgM serum antibodies to glycosylated (native) and deglycosylated murine IgG, using an ELISA system. Serum samples are as follows: P, healthy individual (preexisting response); R1, R2, and R3, patients with active rheumatoid arthritis (polyclonal rheumatoid factors).

Fig. 6. Elevated human IgM anti-murine IgG response in sera from three patients with rheumatoid arthritis (samples R1, R2, and R3) compared to the preexisting IgM anti-murine IgG response of a healthy control (sample P), ELISA system.

Fig. 7. Typical change in response to murine IgG (A) and human IgG (B) in the serum from patients before (pre) and after (post) therapeutic administration of 131I-labeled murine monoclonal antibodies, ELISA system.

displays a high degree of reactivity with both human and murine IgG. The patient from which this polyclonal rheumatoid factor had been isolated had no known prior contact with murine proteins. Binding of this purified polyclonal rheumatoid factor was greater to human IgG than to murine IgG.

Binding of Purified Monoclonal Rheumatoid Factor to Murine and Human IgG. As with the other experiments half a 96-well microtiter plate was coated with murine IgG and the other half with human IgG.

In rows 1, 2, 7, and 8 of the microtiter plate only PBS-Tween was added to ensure that the second layer antibody (anti-human-μ-chain peroxidase-conjugated antibody) did not react with the gamma chain of either murine or human IgG. In rows 3, 4, 9, and 10 serial dilutions of a blood donor’s serum was made. In rows 5, 6, 11, and 12 serial dilutions of purified monoclonal rheumatoid factor were made, starting at a concentration of 10 μg/ml. The diluent for the serum and monoclonal rheumatoid factor was PBS + 0.05% Tween 20.

As can be seen in Fig. 3, unlike the polyclonal rheumatoid factor, the monoclonal rheumatoid factor did not recognize any determinants on the murine IgG. This absence of binding to murine IgG was shown for all four purified monoclonal rheumatoid factors tested.

Antidiotype Antibodies Inhibit Binding of Monoclonal Rheumatoid Factor to Human IgG. In order to determine that the binding of monoclonal rheumatoid factors was a specific interaction between the binding site of the rheumatoid factor and antigenic determinants on the human IgG, and not due to Fc-Fc interaction, we preincubated overnight at 4°C the monoclo-
which had been coated with human IgG (500 ng/well). As antiidiotype monoclonal were used at 10 Mg/ml. body C8E3. Both monoclonal rheumatoid factor and blocking nal rheumatoid factor with the preexisting anti-murine monoclonal anti-body C8E3. Both monoclonal rheumatoid factor and blocking antiidiotypic monoclonal antibody were used at 10 µg/ml.

Binding of the rheumatoid factor was then assayed on a plate which had been coated with human IgG (500 ng/well). As shown in Fig. 4, 80% binding was inhibited by C8E3 indicating that the rheumatoid factor/human IgG interaction is specific, rather than due to nonspecific (Fc-Fc) interactions. Binding was not inhibited by an irrelevant monoclonal antibody of the same isotype.

Binding of the Preexisting Anti-Mouse Immunoglobulin Antibodies and Rheumatoid Factors to Glycosylated and Deglycosylated Murine Monoclonal Antibodies. In order to determine whether the antigenic determinants on the murine immunoglobulin which were being recognized in the preexisting anti-murine immunoglobulin response and by the rheumatoid factors, were protein or sugars, we deglycosylated two murine IgG antibodies.

In order to compare the preexisting IgM anti-mouse response and the response of rheumatoid factors to the intact and deglycosylated antibody a 96-well microtiter plate was coated as follows. In rows 1 to 6 500 ng/well of the antibody HMFG1 (IgG1) was coated, while rows 7 to 12 were coated with 500 ng/well of the deglycosylated HMFG1. This enabled a comparison of the IgM response under identical experimental conditions.

As can be seen in Fig. 5 there was no significant change in response to the deglycosylated antibody by rheumatoid factors. In fact, of all serum samples and purified rheumatoid factors tested (Table 1), one blood donor consistently gave an increase in response to the deglycosylated antibody when his serum was assayed. All others showed essentially no difference (Fig. 5). Similar data were also obtained from another IgGl murine immunoglobulin response and by the rheumatoid factors.

Patients with Active Rheumatoid Disease. Since patients' pretherapy serum, blood donors' serum, and purified polyclonal rheumatoid factor all bind to both human and murine IgG (Figs. 1 and 2), it was of interest to study the relative amounts of anti-murine Ig antibody activity in normal/pretherapy serum versus serum levels in patients with active rheumatoid disease.

From Fig. 6 it is clear that in those patients with active rheumatoid disease (elevated levels of rheumatoid factor), the level of human IgM anti-murine IgG antibody response is correspondingly elevated. To the best of our knowledge these patients had never been immunized with murine immunoglobulin, either actively or passively.

Elevated Human IgM Response to Both Murine and Human IgG in Patients Treated with Radiolabelled Murine Monoclonal Antibodies. Our next question was, did the opposite occur? Do patients treated with a single therapeutic dose of radiolabelled murine monoclonal antibody develop an elevated human IgM anti-human IgG response in parallel to their increase in human anti-murine IgG response?

The results shown in Fig. 7 are for a patient with ovarian cancer treated with 10 mg of radiolabelled murine monoclonal antibody and are representative of those obtained for 12 other patients (Table 2) similarly treated.

Fig. 7 shows that there was an elevated IgM response to both murine and human IgG. The increase in response to murine IgG was greater than that to human IgG.

### DISCUSSION

One of the main problems associated with the in vivo use of murine monoclonal antibodies is the development of the human anti-mouse immunoglobulin response. We and others have previously reported (30-33) that the majority of patients and healthy controls possess preexisting anti-murine immunoglobulin antibodies, mainly of the IgM class, and that the antigenic determinants to which they bind are located on the Fc portion of the murine immunoglobulins. Since rheumatoid factors consist predominantly of IgM antibodies that are directed against the Fc (γ-2 domain) of human IgG, we have investigated the relationship between these two antibody activities.

In this study we demonstrate that IgM preexisting human anti-mouse Ig antibodies and polyclonal rheumatoid factors bind to both human and murine immunoglobulin (Table 1, Figs. 1 and 2). Since the major part of the binding of rheumatoid factors to human IgG and of the preexisting antibodies to mouse IgG can be blocked by preabsorption with mouse and human IgG, respectively, we conclude that the majority of antibody molecules in these sera have specificity for evolutionarily conserved antigenic determinants that are shared by the IgG (Fc) immunoglobulins of man and mouse. Only a small component appears to be against species specific determinants.

In contrast, monoclonal rheumatoid factors isolated from four patients with mixed essential cryoglobulinaemia bound only to human IgG (Fig. 3). However, it is possible that if a larger number of monoclonal rheumatoid factors were studied, then one or more might be found to react with murine as well as human IgG. The specificity of the binding that we detected between IgM antibodies (rheumatoid factors and preexisting antimouse) and target IgG was demonstrated by the ability of antidiotypic antibody C8E3 to block the binding of monoclonal rheumatoid factors to human IgG (Fig. 4). Our ELISA system is therefore detecting specific antibody-antigen binding and not an artificial Fc-Fc association. The 20% activity that is not blocked results from the fact that in the assay the lower affinity binding between soluble antiidiotype and rheumatoid factors is competing with the higher affinity binding of rheumatoid factors with the solid phase IgG on the ELISA plate.

From our comparison of the binding of sera and purified rheumatoid factors (data not shown) to glycosylated and deglycosylated murine IgG (Fig. 5) we conclude that essentially all the antibody activity of preexisting anti-mouse immunoglobulin antibodies and of polyclonal rheumatoid factors is directed against protein rather than sugar determinants. These antibodies therefore cannot be classified as typical heterophile antibod-

<table>
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<tr>
<th>Patient</th>
<th>Binding to murine IgG</th>
<th>Binding to human IgG</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
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</tr>
<tr>
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<tr>
<td>5</td>
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<tr>
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</tr>
<tr>
<td>13</td>
<td>0.367</td>
<td>1.729</td>
</tr>
</tbody>
</table>

* Data for this patient is also presented graphically (Fig. 7).
ies (IgM antibodies that bind to carbohydrate determinants and hence show broad cross-reactivity on many glycosylated molecules). We demonstrate that sera from patients with active rheumatoid arthritis have raised levels of polyclonal rheumatoid factors with increased binding to both human and murine IgG. This is presumably due to a boosting of the immune response to shared antigenic determinants via autoantigenic human IgG. The resulting elevated antibody activity against human and murine IgG may arise from an expansion of existing B cell clones, stimulation of additional B cells producing antibodies to a wider range of antigenic determinants on IgG, or from an increase in the affinity of antibodies produced via selective stimulation of high affinity B lymphocytes.

Although all of our studies have looked at antibody binding in vitro, using an ELISA detection system, where the antibody binding to antigen fixed on a solid surface is probably in the order of two to three logs greater than binding to soluble antigen, the clinical implication of our findings are 2-fold: Firstly, as rheumatoid factors, which bind to antigenic determinants located in the Fc region of the murine monoclonal antibody, are present in a high percentage of the population at subclinical levels, our results would favor the use of F(ab')2 fragments rather than intact IgG for in vivo tumor localization studies, which could possibly result in a higher percentage of the administered dose being targeted to the tumor.

Secondly, patients with active rheumatoid disease were shown to have elevated IgM anti-mouse immunoglobulin antibodies. This, combined with the elevation in anti-human IgG antibodies in patients treated with radiolabeled murine monoclonal antibodies, leads us to conclude that patients with elevated serum rheumatoid factors could, if in vivo binding to the administered murine monoclonal antibodies occurred, be at higher risk of developing immune complex disease.

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REFERENCES


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