Human immunological response to mouse monoclonal antibodies in the treatment or diagnosis of malignant diseases

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Received 8 March 1988

Summary

An overview of the literature is presented concerning the formation, detection, incidence and effect of the human immunoglobulin response on immunoscintigraphy. The following conclusions are drawn. The production of human anti-mouse antibodies (HAMAs) is associated with a diminished therapeutic response; adequate prevention of HAMA production is not yet possible; high HAMA titres give rise to rapid clearance of MoAb into the liver and marked reduction of tumour uptake which results in reduced image quality on immunoscintigraphy; alteration of antibody biodistribution is likely to be related to the in vivo formation of antibody-antibody complexes which could interfere with image quality when sequential imaging is carried out.

Introduction

Monoclonal antibodies have been used for more than a decade in biomedical research. One of the most exciting and promising areas of research is the use of specific monoclonal antibody radionuclide conjugates for diagnostic imaging (immunoscintigraphy) and therapy for malignant diseases. When these monoclonal antibodies (MoAbs), most of which are developed from mouse hybridomas, are injected into the patient, they are recognized as foreign globulins.

The resulting immune response leads to the development of human anti-mouse antibodies (HAMAs), which can be of practical significance. Once HAMAs have been induced, they are able to neutralize the effects of the MoAbs. Since repeated injections lead to rising HAMA concentrations, the efficacy of this approach may be short-lived. As this is regarded as a major complication of the use of MoAbs for clinical purposes, it is essential to establish the scope of the problem of the production of HAMAs.
This paper attempts to present an overview of the literature concerning the formation, detection and incidence as well as the effect of the human immunoglobulin antibody response on immunoscintigraphy.

Findings in the literature

**Human anti-mouse antibodies (HAMAs)**

When a normal individual is exposed for the first time to a foreign antigen, there is a lag phase that may last as long as 10–12 days before antibodies appear in the serum. This primary immune response consists in general of IgM antibodies. Subsequent encounters with the same antigen usually evoke an enhanced secondary or memory response characterized by marked production of IgG [1].

Every antibody has fundamentally the same structure in that it consists of a heavy and a light chain; it also contains a variable and a constant region which may act as antigenic determinants. The antigenic constituents of the variable region of an immunoglobulin are known as its idiotype. The part of the variable region which forms its specific binding site is called its paratope. Thus, it is possible to distinguish between anti-idiotypes directed against idiotypes within the binding site (anti-paratopic) and those directed against idiotypes outside the binding site. Only those binding to the antigen-binding site inhibit the interaction between that binding site and the antigen. Antibodies directed against the constant region are called anti-isotopic antibodies.

Jerne [2] postulated a network of interacting antibody molecules and lymphocytes in which idiotypes of antibody molecules are recognized by anti-idiotopic (AB2) antibodies. This AB2 response is probably a very important part of the human immunological response. The immune system may be regulated at least in part by a network of interactions between idiotypes and anti-idiotypes. He also suggested that AB2 antibodies may exert a strong inhibitory effect on B cell clones during the immune response. Of interest is the fact that injection of these AB2 antibodies into the patient can also give rise to HAMAs [3]. Moreover, AB2 antibodies already present in the body have been shown to be potent enhancers or inhibitors of the immune system.

It is known that healthy individuals possess antibodies against various animal proteins [4] and the patients with various malignancies are able to produce a range of antibodies [5]. There are many reports in the literature concerning the presence of pre-treatment anti-mouse antibodies [6–15]. Naturally occurring anti-mouse activity was demonstrated in the serum of 990 of 1008 healthy blood donors by Thompson et al. [10]. The aetiology of these pre-existing HAMA levels could be vaccination in the past, animal handling or dietary exposure. Another plausible explanation came from Shawler et al. [16], who suggested that such HAMA levels are probably related to the sensitivity of the assay and represent nothing more than background levels caused by non-specific human immunoglobulin. The HAMA levels found by Ritter et al. [17] in normal serum were equivalent to the level of endogenous anti-human immunoglobulin (rheumatoid factor) also found in normal serum [18]. It is therefore
reasonable to suppose that pre-existing HAMA levels may merely reflect a facet of the normal immune system. The question of when an HAMA level should be considered indicative of HAMA production should therefore be dependent upon the upper limit of normal. Carrasquillo et al. [19] consider a patient HAMA-positive when the percentage binding is at least 3 s.d. greater than the mean for normal individuals. The variety of methods and techniques currently in use for detecting the human anti-mouse antibody response in serum, i.e. radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), haemagglutination tests (HAT) and immunofluorescence assays (IF), makes it difficult to estimate the mean value for normal individuals.

According to the literature, HAMAs are first detected after day 2 [20]. However, the moment of first detection is highly variable, as illustrated by Goodman et al. [14] who even found the first detectable HAMA level 233 days after treatment. Therefore, in other studies more patients might have been found to be HAMA-positive if serum samples had been taken at a later stage. The antiglobulin response consists mainly of IgG antibodies, although IgM antibodies have also been observed [7, 9, 11, 15, 21–23]. The rapid elevation of the antiglobulin level reported by several authors [7, 9, 11, 12, 24, 25] is consistent with the kinetics of a secondary immune response, but in general HAMA production occurs 2–3 weeks after MoAb injection; the levels subsequently decrease gradually in the course of several weeks. However, as mentioned above, HAMAs have been detected for up to 300 days after MoAb administration; in fact, in one case an AB2 response persisted [26] for more than 770 days. The fact that antiglobulin levels do not recur indicates that feedback inhibition of the globulin response probably does not occur [9].

The first investigations of the specificity of the antiglobulin response suggested that the response was directed mainly (95%) against the constant region of the MoAb (anti-isotopic), while a minority of the antibodies was directed against the variable region (anti-idiotopic) of the MoAb [7, 11, 27]. However, in man, 50% of the patients receiving the 17-1A monoclonal antibody against a colon carcinoma antigen exhibited an anti-idiotopic AB2 response [28]. Recently, more authors have found that a relatively high percentage of the responses is anti-idiotopic [14, 15, 26, 29]. The difference in results is not yet understood. It is possible that a large percentage of AB2 is followed by anti-anti-idiotopic antibodies (AB3), which could hamper detection of the AB2; another explanation is that the AB2 response is dependent on the type of MoAb. Shawler et al. [15] suggest that multiple infusions of a single MoAb will result in a marked specific response, while infusion of two or more monoclonal antibodies may induce only anti-isotopic antibodies.

Variables which influence the development of human anti-mouse antibodies

Of primary interest is the group of variables that determine why some individuals develop an antiglobulin response during immunoscintigraphy or immunotherapy while many others do not. Shawler et al. [15] were unable to correlate the lack of response to a large number of clinical parameters, and it still remains difficult to
predict on the basis of clinical data which patients will develop antibodies. Although
the development of HAMAs is not related to skin-test positivity [8, 11], the outcome
of lymphoproliferative assays [30] or previous therapy, there are some variables that
presumably do influence HAMA production.

Table 1. Incidence of HAMAs in patients receiving labelled antibodies for
immunoscinctigraphy.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>MoAb</th>
<th>Pat/HAMA*</th>
<th>Frequency(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larson et al. [38]</td>
<td>1983</td>
<td>96.5</td>
<td>6/3</td>
<td>50</td>
</tr>
<tr>
<td>Carrasquillo et al. [50]</td>
<td>1983</td>
<td>96.5</td>
<td>3/3</td>
<td>100</td>
</tr>
<tr>
<td>Reynolds et al. [51]</td>
<td>1985</td>
<td>96.5</td>
<td>37/12</td>
<td>32</td>
</tr>
<tr>
<td>Engelstad et al. [22]</td>
<td>1986</td>
<td>96.5</td>
<td>6/3</td>
<td>50</td>
</tr>
<tr>
<td>Reynolds et al. [32]</td>
<td>1986</td>
<td>T101</td>
<td>20/6</td>
<td>30</td>
</tr>
<tr>
<td>Carrasquillo et al. [52]</td>
<td>1987</td>
<td>T101</td>
<td>4/0</td>
<td>0</td>
</tr>
<tr>
<td>Rosen et al. [21]</td>
<td>1987</td>
<td>T101</td>
<td>6/6</td>
<td>100</td>
</tr>
<tr>
<td>Reynolds et al. [32]</td>
<td>1986</td>
<td>B72.3</td>
<td>30/15</td>
<td>50</td>
</tr>
<tr>
<td>Murray et al. [13]</td>
<td>1987</td>
<td>ZME018</td>
<td>17/7</td>
<td>41</td>
</tr>
</tbody>
</table>

*No. of evaluable patients in the study/Incidence of HAMAs.

In the first place, it has been observed [31] that HAMAs are seldom encountered
in patients with B cell malignancies but are frequently found in patients with T-cell
or solid tumours. The variation in the incidence of HAMAs (see Tables 1 and 2)
probably depends on the immunocompetence of the subjects. This is illustrated by
the fact that out of six patients with chronic lymphocytic leukaemia none exhibited
an immune response to MoAb T101 (an anti-human T-cell monoclonal antibody)
whereas five out of ten patients with cutaneous T-cell lymphoma had measurable
HAMA activity [15]. Moreover, the immune system of patients with less advanced
disease might be expected to be more competent so that the likelihood that HAMAs
will develop would be greater. Theoretically, it is feasible that healthy humans should
have a 100% response rate to murine MoAbs. In the second place, mouse whole
antibody is more immunogenic than the Fab fragment [32]. However, it has been
shown that repeated administration of the murine Fab fragment will also lead to a
high frequency of HAMA positivity [15, 19, 20, 24, 26]. In addition, the development
of HAMAs may be dose-dependent [9, 20, 21, 28, 30, 33]. Eight out of nine patients
who were given less than 200 mg MoAb developed HAMAs compared with only one
out of nine receiving higher doses, suggesting that larger doses of MoAb could induce
tolerance for murine immunoglobulin [20]. Essentially the same observation was re­
ported by Oldham et al. [9], who found measurable increases in anti-globulin response
after administration of 50 mg doses and a loss of demonstrable antiglobulin at higher
doses. Herlyn et al. [26] were not able to confirm this correlation but found instead
### Table 2. Incidence of HAMAs in patients undergoing immunotherapy.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>MoAb</th>
<th>Pat/HAMA*</th>
<th>Frequency (%)</th>
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<tr>
<td>Miller et al. [53]</td>
<td>1981</td>
<td>Leu-1</td>
<td>1/1</td>
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<td>Miller et al. [7]</td>
<td>1981</td>
<td>Leu-1</td>
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<td>1983</td>
<td>Leu-1</td>
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<td>57</td>
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<td>1982</td>
<td>17-1A</td>
<td>4/3</td>
<td>75</td>
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<tr>
<td>Koprowski et al. [28]</td>
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<td>17-1A</td>
<td>29/10</td>
<td>34</td>
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<tr>
<td>Sears et al. [20]</td>
<td>1984</td>
<td>17-1A</td>
<td>18/9</td>
<td>50</td>
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<tr>
<td>Sears et al. [29]</td>
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<td>17-1A</td>
<td>20/10</td>
<td>50</td>
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<td>50</td>
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<td>17-1A</td>
<td>37/32</td>
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<td>1986</td>
<td>17-1A</td>
<td>65/35</td>
<td>54</td>
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<tr>
<td>Sindelar et al. [25]</td>
<td>1986</td>
<td>17-1A</td>
<td>25/23</td>
<td>92</td>
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<td>Lobuglio et al. [39]</td>
<td>1986</td>
<td>17-1A</td>
<td>20/17</td>
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<td>Frödin et al. [23]</td>
<td>1986</td>
<td>17-1A</td>
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<td>100</td>
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<td>Douillard et al. [60]</td>
<td>1986</td>
<td>17-1A</td>
<td>20/11</td>
<td>55</td>
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<td>Steplewski et al. [61]</td>
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<td>1982</td>
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<td>11/5</td>
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<td>Linch et al. [37]</td>
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<td>Carrasquillo et al. [19]</td>
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<td>48.7</td>
<td>8/5</td>
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<td>Goodman et al. [14]</td>
<td>1985</td>
<td>96.5; 48.7</td>
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<td>9.2.27</td>
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<td>Houghton et al. [59]</td>
<td>1985</td>
<td>R24</td>
<td>12/12</td>
<td>100</td>
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<td>Press et al. [31]</td>
<td>1987</td>
<td>1F5</td>
<td>4/1</td>
<td>25</td>
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</table>

*No. of evaluable patients in the study/Incidence of HAMAs.

A positive correlation between the number of injections and the occurrence of HAMAs. In addition to the dose of MoAb and the number of injections, the time interval between injections (treatment schedule) has also been associated [19, 20] with HAMA production. Finally, differences in response may be related to pre-existing antiglobulin level [11] or the route of administration. Reynolds et al. [32] suggest that there is no apparent difference in HAMA development when an antibody is given subcutaneously.
or intravenously. It is interesting that interstitial administration of MoAbs increased anti-mouse activity [22], whereas a similar dose of the MoAb given intravenously could not be associated with increasing HAMA levels.

**Effect of human anti-mouse antibodies**

Radioimmunotherapy studies indicate that immune complexes may form in the presence of circulating free antigen. HAMA may also form complexes with the injected MoAb, probably in direct proportion to the HAMA concentration [34, 35]. On the other hand, Oldham et al. [9] showed that only one out of eight patients exhibited HAMA-MoAb complexes. The consequences of complex formation, should it occur, are likely to depend upon the patient, the type of malignancy and the MoAb used. The formation of complexes of MoAbs and the circulating target cells has been associated with serum sickness, renal toxicity and various adverse reactions that could inhibit effective antibody therapy or imaging [9]. Although Bertram et al. [30] described a patient with an anaphylactoid reaction associated with the development of HAMAs, it should be noted that patients with HAMAs generally exhibit no toxicity whatsoever, presumably because of the blocking effect of the antibody [36, 37].

The bioavailability of a MoAb for a tumour depends on the MoAb dose, the levels of circulating antigen and antigen expression of the tumour, the kinetics of antigen modulation and the presence of HAMAs. It has been demonstrated by Linch et al. [37] that therapeutic failure is attributable to the appearance of anti-mouse antibodies and not to antigenic modulation. The development of antibodies against mouse immunoglobulin led to a marked decrease in the levels of circulating mouse immunoglobulin [24, 39]. Free antibodies could no longer be detected after the development of HAMAs [27]. HAMAs are able to consume antibodies, thus preventing the binding of these antibodies to cellular antigens [3, 15, 24, 27, 36, 37]. It is therefore obvious that anti-mouse antibodies eventually neutralize the therapeutic effect of MoAbs. Once an immune response begins, further infusions of antibody are not capable of inducing tumour regression. However, the results of Oldham et al. [9] indicate that murine antibody can be given in repeated doses to immunologically intact patients with a solid tumour without eliciting a therapeutically limiting anti-globulin response. Miller et al. [27] showed that host antibodies could block the binding of anti-Leu-1 to target cells *in vitro*. Similarly, host antibodies may neutralize the *in vivo* and *in vitro* effects of MoAbs, probably by increasing the removal of these antibodies and/or by blocking.

Some MoAb clinical trials have led to the suggestion that an immune response against the murine immunoglobulin could be beneficial [28]. This is based on the report of Miller et al. [40], who described very successful treatment of a patient with progressive lymphocytic lymphoma, and on the theoretical consideration that second (AB2) and third (AB3) order antibodies with tumour-binding activities will ultimately induce an immune attack by the host against the tumour. It is possible that the AB2 antibody will trigger an active anti-tumour response in the regulatory immune network.
Human immunological response to mouse monoclonal antibodies

It has been reported that the anti-idiotopic response correlates with clinical responses [3, 26, 28, 40], but according to Lowder et al. [41] and Sindelar et al. [25] there is no relation between AB3 and clinical response. While anti-idiotypes may be used in the future to advantage for therapeutic purposes, they also limit the more straightforward use of mouse antibodies for immunosuppression.

The production of HAMAs will cause changes in the pharmacokinetic behaviour of MoAbs. It is postulated that HAMAs stimulate the removal of MoAbs by the reticuloendothelial system. Patients with detectable HAMA levels cleared the labelled mouse antibody much more rapidly than those without HAMAs [27, 38]. Rosen et al. [21] reported that the HAMA response is most probably responsible for the enhanced antibody clearance rates seen after retreatment. This effect has been observed using radiolabelled MoAbs. Radiolabelled MoAbs are currently in use for diagnostic imaging of tumours. Low doses (< 10 mg) of MoAbs are labelled with radioisotopes suited for external gamma scintigraphy. In most cases a single injection of the radiolabelled MoAb is adequate for tumour imaging. However, when sequential imaging is performed multiple injections are necessary. In that case the uptake in the liver can be significantly higher on the second occasion and the tumour uptake could be less marked [38] which could result in reduced image quality [42-45].

Another effect, which has been described extensively [46], is positive interference of the HAMAs with the effectiveness of two-site immunoassays. These artefacts have been observed in both monoclonal and polyclonal sandwich-type immunoassays.

Prevention of human anti-mouse antibodies

Many investigators hope that human monoclonal antibodies or recombinant chimeric antibodies, obtained by chemical coupling of the mouse variable region to the human constant region by genetic engineering, will be the key to preventing the immunogenicity associated with immunoglobulins. Although it has been suggested that human monoclonal antibodies could reduce the immune response, the work of Shawler et al. [15] implies that such antibodies might still induce anti-idiotype antibodies. The immunogenicity in man of human monoclonal as well as chimeric antibodies has not yet been tested.

Because of the relationship between immunocompetence and the likelihood of HAMA development, Miller et al. [27] tried to prevent the occurrence of HAMAs by giving cyclophosphamide during MoAb treatment. The result was not successful. The use of concurrent therapies, such as chemotherapy [47], radiotherapy or immunosuppressive drugs [48], to suppress the response or produce immunologic tolerance has had some effect in reducing HAMA levels. The initial administration of large doses of highly purified monoclonal antibodies might induce tolerance [20], while the proper dose and treatment schedule could mimic the effects of HAMA production [21, 30]. Goodman et al. [14] reported that a loading dose, that produces high plasma concentrations, followed by a maintenance dose every 48 h resulted in a lack of HAMA development. The application of plasmapheresis or affinity columns to lower the
amounts of free antigen or eliminate the HAMAs formed [3, 7, 19, 21, 49] has been reported to be successful [21]. Other more speculative approaches to the prevention of HAMA development are skin antigen desensitization and, theoretically, the use of immunoconjugates with toxin, radioactivity or drug molecules that would bind to and destroy the HAMA-producing B cells [15].

Conclusions

Analysis of the factors that play an important role in the development of HAMAs is complicated by such problems as the relatively small number of reports, the variety of diseases treated and the lack of uniformity in the design of these trials. This makes it difficult to predict the type of host anti-mouse immunoglobulin response to be expected. Although data regarding HAMA production are limited, some conclusions can be drawn.

First, the production of HAMAs is associated with a diminished therapeutic response.

Secondly, adequate prevention of HAMA production is not yet possible. All available methods for prevention, except for that based on an increase in the dose of MoAb, are not very practical at the moment.

Thirdly, as far as immunoscintigraphy is concerned, the most important consequence of high HAMA titres is the rapid clearance of MoAb into the liver and marked reduction of tumour uptake, which results in reduced image quality [38, 42–45].

Fourthly, it is likely that the alteration of antibody biodistribution is related to the in vivo formation of antibody-antibody complexes [35] and that this alteration could interfere with image quality when sequential imaging is performed, even when carried out several months after the initial investigation [38].

Acknowledgement

This paper has been prepared under the auspices of the joint Task Group on Clinical Utility of Labelled Antibodies of the European Association of Nuclear Medicine.

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