Brief Definitive Report

THE IMMUNOGENICITY OF CHIMERIC ANTIBODIES

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Owing to problems in making high affinity human mAbs, there is interest in the therapeutic application of chimeric antibodies in which either the V domains or just the hypervariable regions of rodent mAbs have been used to replace the equivalent parts of a human antibody (1, 2, and references therein). Whereas xenogeneic antibodies are highly immunogenic in man (see reference 3 for references), little is known about the immunogenicity of chimeric antibodies. It is unclear to what extent a particular V domain is characteristic of the species from which it originates, and therefore, whether a response will be elicited by an antibody in which only the V region is foreign. If there is such an antiidiotypic response, to what extent is it enhanced by linkage to foreign C domains? Here, we describe experiments carried out in the mouse that address these questions.

Materials and Methods

Mice and Immunizations. Mice were from Olac, Bicester, UK, or National Institute for Medical Research, Mill Hill, UK. Prebleed sera were taken from 6–8-wk-old females (six per group), which were then injected intraperitoneally with the relevant antibody (40 μg) in CFA. Serum was taken 30 d later, and the animals were boosted intraperitoneally with the same antibody (40 μg) in IFA; serum was taken after a further 10 d. For injection with cell-bound antibody, spleen cells from F_1 mice were conjugated with 4-hydroxy-3-nitrophenacetyl (NP)-kephalin (4); mice were immunized intravenously with 5×10^6 syngeneic NP-spleen cells mixed with 40 μg of anti-NP antibody. The boost (day 30) was the same as the primary immunization.

Antibodies and Immunoassays. Antibodies were purified by affinity chromatography (4) from the supernatants of cells of the J558L plasmacytoma (which secretes λ L chains) transfected with plasmids directing the synthesis of the appropriate antibody H chain. The H chain genes for HuV_{NP}-Huγ2, HuV_{NP}-Moγ2b^b, and MoV_{NP}-Moγ2b^b were assembled by inserting C_H exon fragments (7.2-kb Hind III–Bam HI fragment for human γ2 [described in reference 4]; 4.2-kb Eco RI–Bgl II fragment for mouse γ2b^b [ref. 5]) into the pSV-V_{NP} vector or a derivative containing the HuV_{NP} V domain (2). Other transfectants have been described (1, 2, 4).

Antibody responses were measured by ELISA. Serum dilutions were incubated in microtitre plates coated with the relevant IgH, λ anti-NP antibody. Bound antiantibodies were detected using biotinylated anti-mouse κ antiserum and horseradish peroxidase coupled to streptavidin. Immune sera had less than threefold the prebleed titre of residual λ-bearing anti-NP anti-

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body, as well as of antibodies reacting with either mouse IgM, λ myeloma protein, or purified λ L chains.

Results and Discussion

The response was compared of mice injected with one of three antibodies. The most xenogeneic antibody (HuV_{NP}-Huγ2) is composed of a human γ2 C region linked to a V domain that has the framework residues of the human NEW myeloma protein (Fig. 1). A chimeric derivative (in which only the V region frameworks are human) was created by substituting the human Cγ2 by the Cγ2b of C57BL/6 mice to yield HuV_{NP}-Moγ2b^b. In MoV_{NP}-Moγ2b^b (the syngeneic antibody), the entire V domain is of mouse origin, the foreign framework residues having been substituted by mouse sequences. All the antibodies contain a mouse λ L chain, as well as V_H hypervariable region sequences derived from a mouse antibody specific for NP.

Groups of (C57BL/6 × BALB/c)F_1 mice were immunized intraperitoneally with the three antibodies in CFA. The mice made a strong primary and secondary response to the most xenogeneic antibody, a reduced yet nevertheless considerable response to the chimeric antibody, but no detectable response to the syngeneic antibody (Fig. 2 *A*). In the mice immunized with the HuV_{NP}-Huγ2, a large proportion of the response was directed against the human γ2 C region, as witnessed by binding inhibition assays using a human IgG2 myeloma protein; much less inhibition was given by an antibody (HuV_{NP}-Huε) whose H chain is composed of the HuV_{NP} V_H domain linked to human Cε (Fig. 3 *A*, *I*). The anti-V region response elicited by the xenogeneic antibody HuV_{NP}-Huγ2 was measured using a HuV_{NP}-Huε coat; it was of a similar order to that elicited by HuV_{NP}-Moγ2b^b (Fig. 3 *B*). Thus, a considerable proportion of the response to the xenogeneic antibody was directed against the V region; this antiidiotypic response was not diminished by using the chimeric antibody with self C regions.

The antiidiotypic response in the mice immunized with either HuV_{NP}-Huγ2 or HuV_{NP}-Moγ2b^b was not exclusively directed against the human frameworks of the immunizing antibody, although these are the only foreign determinants in the V domain. The mice contained a significant titre of antibodies that recognized MoV_{NP} (Fig. 3, *A* and *B*). A more direct demonstration that it is possible to elicit an antibody response to syngeneic V domains is provided by immunizing mice with MoV_{NP}-Huγ2 (Fig. 3 *B*). Thus, the mouse can make a response to its own V domains, and probably to the hypervariable regions themselves. However, this response is not elicited unless the administered antibody contains some foreign determinants.

As a better system to mimic the use of mAbs directed against tumor cell surface

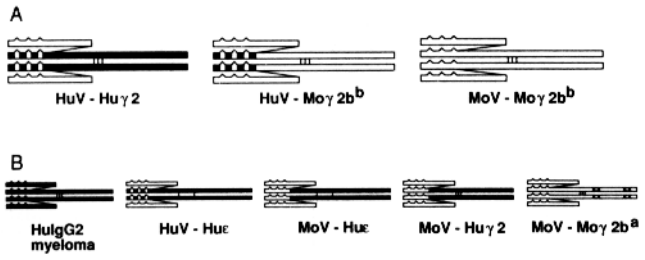

FIGURE 1. Structure of antibodies. (*A*) Antibodies used for immunization. (*B*) Antibodies used for testing the specificity of the responses. The open and filled bars denote sequences of mouse and human origin, respectively. (×) Amino acid positions at which MoV_{NP}-Moγ2b^a and MoV_{NP}-Moγ2b^b differ.

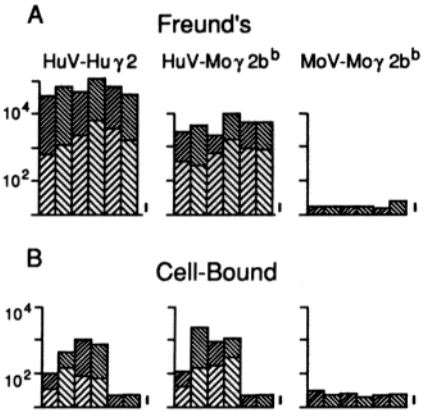

FIGURE 2. Responses to administered antibodies. (*A*) Responses to antibodies emulsified in Freund's. (*B*) Responses to antibodies bound to syngeneic spleen cells. Bars in the histogram give the serum dilution from individual mice that yield half-maximal binding to the immunizing antibody immobilized on the plate. Thus, sera from MoV_{NP}-Moγ2b^b-immunized mice were tested on an MoV_{NP}-Moγ2b^b coat, etc. Lightly crosshatched bars give titres for the primary response; stronger crosshatching indicating the increase in the secondary response. A bar indicates the titres obtained from the preimmune sera. Where there was no significant difference between the primary and secondary responses, only the secondary is depicted.

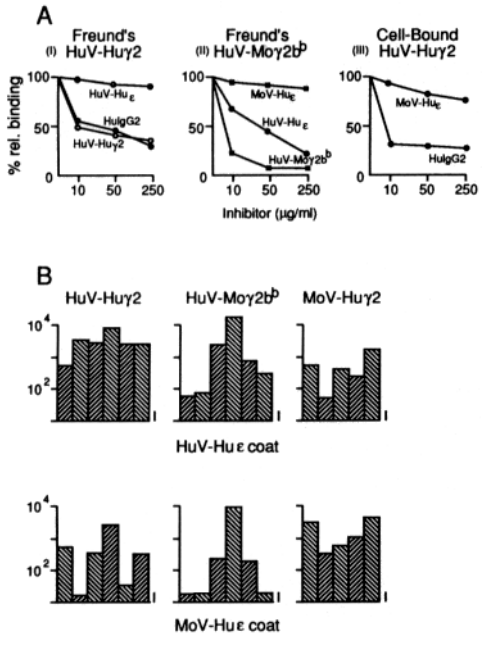

FIGURE 3. Specificity of the antiantibodies. (*A*) Binding inhibition assays. The bindings of antiantibodies in a serum dilution from individual mice hyperimmunized with (*I*) HuV_{NP}-Huγ2 in Freund's, (*II*) HuV_{NP}-Moγ2b^b in Freund's, or (*III*) cell-bound HuV_{NP}-Huγ2 were tested on a coat of the immunizing antibody in the presence of various concentrations of competitor. The result is given as the percentage binding relative to that obtained in the absence of inhibitor. Inhibition assays shown are for individual mice but are representative of the three in each group tested. (*B*) Direct binding of sera from mice hyperimmunized with HuV_{NP}-Huγ2, HuV_{NP}-Moγ2b^b, or MoV_{NP}-Huγ2 antibody in Freund's to a MoV_{NP}-Huε or HuV_{NP}-Huε coat; binding could not be inhibited with a human IgE myeloma protein. Bars for individual mice titred on MoV_{NP}-Huε are aligned with bars for the same mice titred on HuV_{NP}-Huε.

markers, mice were challenged with syngeneic spleen cells to which antibody had been bound. While the responses were considerably weaker than to the antibodies administered in CFA, the cell-bound xenogeneic and chimeric antibodies nevertheless elicited a clear response with the major part of the response to HuV_{NP}-Huγ2 being directed against the C region (Figs. 3 *A* and 2 *B*). Within the variation from individual animals, there was no clear difference in the immunogenicity of the xenogeneic and chimeric antibodies. The contrast between these results and those obtained using Freund's might be accounted for by the fact that mouse IgG2b, but not human IgG2, binds to some mouse Fc receptors (6).

Although administration of a syngeneic antibody need not elicit an antiantibody

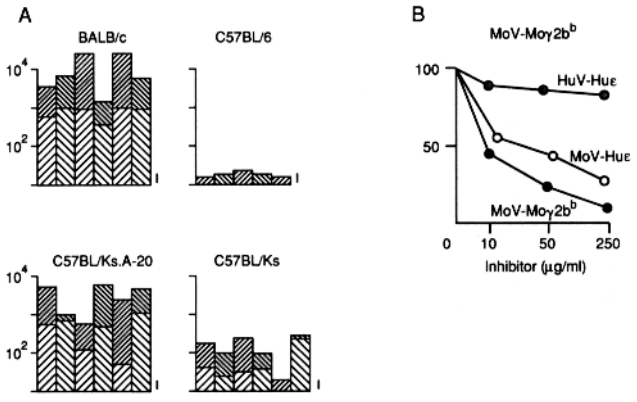

FIGURE 4. Allogeneic responses. (*A*) The response of C57BL/6, BALB/c, C57BL/Ks, and C57BL/Ks.A-20 mice to immunization intraperitoneally with MoV_{NP}-Moγ2b^b antibody in Freund's. (*B*) Binding inhibition assay of the antiantibody response in one of the MoV_{NP}-Moγ2b^b-immunized BALB/c mice. Of three other mice tested, two gave curves similar to those presented, whereas one showed a greater degree of inhibition by MoV_{NP}-γ2b^b than by MoV_{NP}-Hue.

response, polymorphism within the human population may lead to responses even to wholly human antibodies. To compare the magnitude of such an allotypic response with the response mounted against foreign V region frameworks, MoV_{NP}-Moγ2b^b was injected into both C57BL/6 (the strain from which the antibody originates) and BALB/c mice. Unlike C57BL/6, the BALB/c mice made a strong response against MoV_{NP}-Moγ2b^b, recognizing both V and C domains (Fig. 4 *A*). Although immune response genes could well play a role (7), the difference in the response obtained with the C57BL/6, BALB/c, and F_1 mice is likely to be due to the difference in Igh haplotypes. This was confirmed by comparing the responses of C57BL/Ks (H2^d, Igh^b) with C57BL/Ks.A-20 (H2^d, Igh^a) mice (Fig. 4 *B*).

Thus, an antibody with both foreign C_H domains and foreign V_H frameworks was strongly immunogenic, eliciting a response that was largely directed against the C region but with a substantial component against the V. In a chimeric derivative (in which only the V region frameworks are foreign), the anti-C response was abolished but the response to the V remained and was unattenuated. While all foreign framework sequences may not prove equally immunogenic, the results indicate that, short of administering an autologous antibody, therapeutic applications should make use of antibodies in which care has been taken to reduce the V region immunogenicity. However, the immunogenicity of antibodies in which the hypervariable regions are the sole foreign determinants is an unknown quantity and is an important focus for further research. Extrapolating to therapy in man, the results caution that, even with wholly human antibodies, problems may be encountered with allogeneic responses directed against both the V and the C. Ultimately, it may prove advisable not just to use humanized antibodies, but to use antibodies whose allotype is matched to that of the patient.

Summary

Mice were immunized with model xenogeneic (both the V_H frameworks and the C_H domains of human origin), chimeric (just V_H frameworks human), or self antibodies, and the antiantibody responses were dissected. Only the self antibody did not elicit a response. A strong response was elicited by the most xenogeneic antibody with ~90% against the C and ~10% against the V. The anti-V response was not

attenuated in the chimeric antibody, demonstrating that foreign V_H frameworks can be sufficient to lead to a strong antiantibody response. The magnitude of this xenogeneic anti-V_H response was similar to that of the allotypic response elicited by immunizing mice of the Igh^a allotype with an Igh^b antibody. Thus, although chimerization can diminish antiantibody responses, attention should be paid both to V region immunogenicity and to polymorphism.

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