Clin. exp. Immunol. (1980) 39, 449–454.

Production of antibodies to host IgG after transfer of histocompatible cells primed to host allotype

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(*Accepted for publication* 13 *August* 1979)

SUMMARY

A method is described to bring about endogenous production of antibodies to the Fc region of IgG. Mice of one allotype (Ig^b) were immunized against immunoglobulin from a congenic strain of mice bearing another allotype (Ig^a). The Ig^a-primed cells were transferred to congenic recipients bearing the Ig^a allotype and the production of anti-host allotype antibodies by the donor cells measured. Low levels of anti-host allotype antibodies were found in the sera of normal recipients but much greater amounts in recipients pretreated with cyclophosphamide. On ultracentrifugation some of these IgG antibodies sedimented at more than 7S probably because they were complexed with the IgG antigen. It is considered that this model may be of use in testing the effect of anti-Fc antibodies on joint inflammation.

INTRODUCTION

The salient immunological abnormality in patients with rheumatoid arthritis is the presence in their serum and joint fluid of raised levels of anti-γ-globulins. These antibodies are directed to exposed determinant(s) in the Fc region of immunoglobulins (Natvig & Turner, 1970; Gaarder & Natvig, 1970; Hunneyball & Stanworth, 1976a, b). It is well known that such rheumatoid factors of the IgM isotype are to be found in 70–80% of patients with rheumatoid arthritis, but it has been reported recently that IgG anti-γ-globulins also occur in virtually all patients with the disease (Hay, Nineham & Roitt, 1975; Carson *et al.*, 1977). They appear to be autoantibodies since immune complexes have been found in synovial fluid which are composed mainly, if not entirely, of the patient's immunoglobulin combined with the anti-γ-globulin (Winchester, Agnello & Kunkel, 1970). The role of rheumatoid factors, particularly those of IgG isotype, in the pathogenesis of rheumatoid arthritis is currently debated.

Arthritis can be induced in experimental animals in a number of ways (for review see Imrie, 1976) which appear to be independent of autoanti-γ-globulins. It follows either that autoanti-γ-globulins are not involved in the arthritis of rheumatoid arthritis or that these models do not mimic rheumatoid arthritis in that they lack high levels of autoanti-γ-globulins. In order to distinguish between these alternatives it is necessary to be able to induce autoantibodies to exposed epitopes in the Fc region of immunoglobulins. If this can be achieved then it will be possible to test whether autoanti-γ-globulins are involved under any circumstances in an arthritic process.

We have available in our laboratory two strains of mice, CBA/H and CBA/H/Ig^b (referred to as Ig^b mice), which are congenic except at the Ig-1 allotype locus and probably also the other Ig loci: CBA mice having the 'a' allele for each of the immunoglobulin classes and Ig^b mice the 'b' allele. The allotypic determinants controlled by the Ig-1 locus are situated in the Fc region of mouse IgG2a (Mishell & Fahey, 1964; Herzenberg, Warner & Herzenberg, 1965). Thus the IgG2a molecules of CBA and Ig^b

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0099-9104/80/0200-0449 $02.00

mice are identical except for a few determinants in their Fc region. In addition, these mice probably differ at the Ig-4 allotype locus which controls allotypic determinants in the Fc of the IgG1 isotype (Minna, Iverson & Herzenberg, 1967) since anti-Ig^a allotype sera raised in Ig^b mice react with a purified IgG1 myeloma protein (Elson, unpublished observations).

Ig^b mice can be immunized so that they make high levels of IgG antibodies to immunoglobulins bearing the 'a' allotype (see below). If lymphoid cells from such mice are transferred to CBA mice the donor cells will not be rejected by an allograft reaction because they are histocompatible with the recipient (Micklem *et al.*, 1976; Elson, Jablonska & Taylor, 1976). If the cells were to continue making antibody to the host's allotype the result would be similar to an autoanti-γ-globulin response to determinants in the Fc region of the host's immunoglobulin. This report describes the detection of such a response and a means of increasing it.

MATERIALS AND METHODS

Animals and immunization. CBA/H mice and the congenic Ig^b strain aged between 3–6 months were used. Donor Ig^b mice were immunized against the Ig^a allotype by intraperitoneal injection of CBA(Ig^a) anti-*Bordetella pertussis* antibody coated onto *B. pertussis* (Dresser & Wortis, 1967). Each inoculum contained 2×10^9 antibody-coated organisms.

Antibody assay. The method was similar to that described previously (Elson & Taylor, 1974). Ig^aIgG was attached to polystyrene tubes (LP2: Luckham) by adding 100 μl of a solution of 100 μg/ml IgG in phosphate buffered saline to the tubes and incubating for 2 hr at 37° and overnight at 4°. After washing with PBS, any free reactive sites were blocked by a further incubation for 2 hr at 37° with diluent (1% sheep serum in phosphate buffered saline pH 7·2). Test sera, standard antibody and control sera were diluted in the tubes and incubated at 37° for 2 hr. The tubes were washed with diluent, incubated overnight at room temperature with ^{125}I-labelled purified anti-Ig^b allotype antibody, washed again and counts made of the radioactivity bound to the tubes. By comparison with the results obtained with standard purified anti-DNP and DNP-human gammaglobulin-coated tubes it was possible to estimate changes in Ig^b antibody concentrations in test sera in terms of μg/ml.

Preparation of Ig^a IgG. Sodium sulphate was added to CBA serum to 20% and the resultant precipitate washed twice in 20% sodium sulphate. The precipitate was dissolved and dialysed against 0·01 M HCl-Tris pH 8·5. It was eluted from DEAE sepharose (Pharmacia) with a gradient from 0 to 0·5 M NaCl in 0·01 M HCl-Tris. The IgG, which was contained in the second protein peak (0·13 M NaCl) was collected.

Sucrose density gradient fractionation. This was carried out as described by Jablonska (1977) using 40, 30, 20 and 10% sucrose and taking nine 0·9 ml fractions from the top after centrifugation at 95,000 *g* for 18 hr at 4°. By this method mouse IgG is concentrated in fractions 3 and 4 and mouse IgM in fractions 7 and 8.

RESULTS

Immunization of Ig^b donors

Allotypes are not particularly immunogenic and it was thought necessary to test how many injections of Ig^a antibody-coated pertussis organisms were required to immunize Ig^b mice. Accordingly, groups of Ig^b mice were injected with Ig^a antibody-coated pertussis organisms at weekly intervals; each group receiving a different number of injections. Fig. 1 shows their Ig^b anti-Ig^a response 3 weeks after the final injection and it can be seen that the levels increased steadily up to the fifth injection. It should be emphasized that the Ig^b-bearing antibodies detected are almost entirely IgG since the b allotypic determinants are situated predominantly on IgG (Herzenberg & Herzenberg, 1978; see below). In subsequent experiments donor mice were injected at least five times with antibody-coated pertussis and used between 1 and 3 months after the final injection.

Challenge of Ig^a primed cells

This experiment was designed to reveal whether Ig^a-primed cells responded better to challenge with soluble Ig^a IgG, or to Ig^a antibody-coated pertussis. Irradiated (500 rad) Ig^b mice were each injected intravenously with 2×10^7 Ig^a-primed Ig^b spleen cells and challenged intraperitoneally with an injection of either 100 μg soluble Ig^a IgG, or Ig^a antibody-coated pertussis or left as controls. Eleven days later their Ig^b anti-Ig^a antibody levels in $\log_{10}$ μg/ml were $1{\cdot}15 \pm 1{\cdot}03$, $2{\cdot}04 \pm 0{\cdot}17$ and less than $-1{\cdot}00$ respectively. As the cells challenged with antibody-coated pertussis produced a ten-fold greater response than cells challenged with soluble IgG, the former method was used in the following experiments.

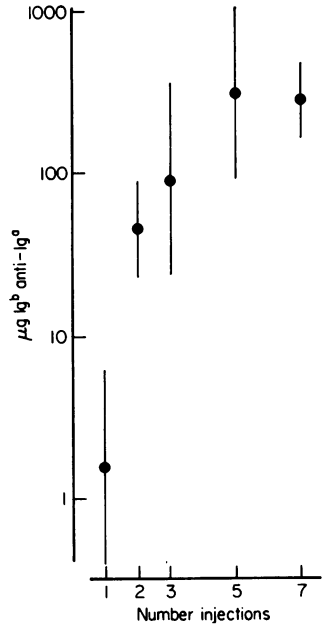

FIG. 1. Anti-Ig^a response of groups of Ig^b mice to various numbers of injections of Ig^a-coated pertussis organisms. The means and standard deviations are shown.

Anti-host allotype response

CBA(Ig^a) mice were each injected intravenously with $7{\cdot}5 \times 10^7$ Ig^a primed Ig^b spleen cells and challenged intraperitoneally with an injection of Ig^a antibody-coated pertussis (day 0). Ig^b anti-Ig^a antibody was not detected in sera taken before transfer and challenge but sera collected afterwards showed small amounts of Ig^b anti-Ig^a activity. The means were $-0{\cdot}33 \pm 0{\cdot}27$, $-0{\cdot}37 \pm 0{\cdot}27$ and $-0{\cdot}49 \pm 0{\cdot}01$ $\log_{10}$ μg/ml of Ig^b anti-Ig^a on days 7, 14 and 29 respectively.

An attempt was made to increase the amount of anti-host allotype antibody produced by the transferred cells by disrupting the host's homeostatic mechanism. CBA mice were given cyclophosphamide

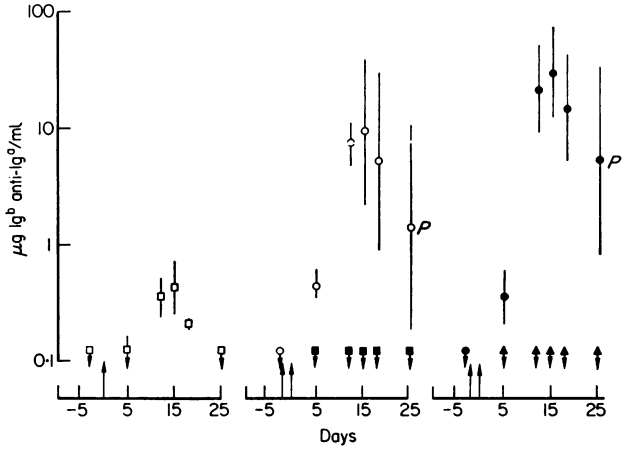

FIG. 2. Anti-Ig^a response of Ig^b spleen cells in CBA (Ig^a) mice. The means and standard deviations are shown. (□) Ig^a primed Ig^b cells in normal mice, (■) normal Ig^b cells and (○) Ig^a-primed Ig^b cells in recipients pretreated with 100 mg/kg body weight cyclophosphamide. (▲) Normal Ig^b cells and (●) Ig^a-primed Ig^b cells in recipients pretreated with 200 mg/kg body weight cyclophosphamide.

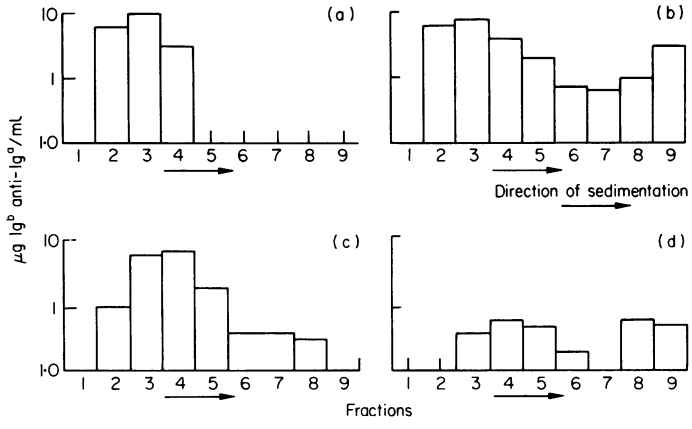

FIG. 3. Anti-Ig^a activity in fractions of sera from (a) an Ig^a-primed Ig^b mouse, (b) and (c) CBA recipients pretreated with cyclophosphamide and given Ig^a primed Ig^b cells, and (d) a normal CBA mouse given Ig^a-primed Ig^b cells.

in doses of either 100 or 200 mg/kg body weight (intraperitoneally) or left untreated. Two days later they were injected intravenously with either Ig^a-primed Ig^b spleen cells or normal Ig^b spleen cells (10^8) and challenged intraperitoneally with Ig^a antibody-coated pertussis (day 0). They were bled at intervals and their sera tested for anti-Ig^a antibodies. The results are summarized in Fig. 2. It is evident that the sera of recipients pretreated with cyclophosphamide and given Ig^a primed cells contained much larger amounts of anti-Ig^a antibody than sera from untreated recipients given Ig^a-primed cells. Cyclophosphamide-treated mice or normal mice (not shown) receiving normal Ig^b spleen cells did not produce any anti-Ig^a antibody.

In order to test if the anti-host allotype activity was free or complexed (presumably with Ig^{a}IgG), sera from the above mice and from the Ig^b donors were subjected to sucrose density gradient fractionation and the fractions tested for anti-Ig^a antibodies. Some of the results are illustrated in Fig. 3. As expected, the Ig^b anti-Ig^a activity in sera from the donor mice sedimented in a single peak in the position previously identified with 7S globulins. This contrasts with the behaviour of the activity in sera of recipient mice which was found both in 7S fractions and in fractions of higher S value.

In a further experiment precipitin tests were set up with an anti-Ig^a allotype serum against dilutions of normal CBA sera and of the anti-host allotype containing sera. Precipitin lines were observed against the anti-host allotype-containing sera showing that they still contained Ig^{a}IgG although the lines were weaker than those obtained against normal CBA sera.

DISCUSSION

We have shown that transferred cells can be made to continue production of antibodies to the host allotype and that such antibodies can be detected in the serum. At first sight this may seem surprising especially when it is considered that molecules reactive with anti-allotype sera can constitute up to 55% of total IgG (Elson *et al.*, 1976). The production of such molecules was not completely inhibited by the transferred cells because they could be detected by the gel-diffusion test. Thus in the presence of both antibody and excess antigen, it was to be expected that much of the antibody should exist in the form of immune complexes. In conditions of antigen excess many of these would be likely to be small and thus not easily distinguished from free antibody. A significant fraction, however, were of larger size and easily resolved on the sucrose gradient. The fact that these antibodies could still be detected by radioimmunoassay in the presence of excess antigen probably depends on the antibody binding with higher avidity to the antigen on the tubes than to the free antigen, by virtue of the opportunity offered by the former for multivalent attachment. A similar consideration must apply when such methods are used to detect rheumatoid factor.

If antigen-primed lymphoid cells are transferred to normal recipients they make a very poor adoptive response unless the recipients' homeostatic mechanisms are somehow disrupted (Dresser, 1961; Celeda, 1966; Bell & Shand, 1975; Feldbush, 1976). Similarly, the response of trinitrophenyl (TNP) primed Ig^b lymphoid cells to alum-precipitated TNP-proteins with pertussis is much greater in lightly-irradiated than normal CBA recipients (Elson & Taylor, unpublished observations). It seems likely that in the present experiments the response of Ig^a-primed cells in CBA mice could be restricted in the same way. This possibility gains credence from the observation that the anti-Ig^a response was enhanced in recipients pretreated with cyclophosphamide. There is evidence that this drug selectively affects suppressor cells. For example, if guinea-pigs are given cyclophosphamide 3 days before sensitization with either ovalbumin or 2,4-dinotrofluorobenzene, then Jones–Mote or contact skin reactions elicited 1 week later are increased in intensity, and prolonged as compared to controls (Turk, Parker & Poulter, 1972; Turk & Parker, 1973).

So far we have observed no pathological effect of the anti-host γ-globulins. The same is reported to be true for another group of anti-globulins: rheumatoid factors. Thus, normal individuals transferred with rheumatoid factor-containing serum do not develop arthritis (Harris & Vaughan 1961), and high levels of both IgM and IgG rheumatoid factor occur in the sera of patients with a variety of infections without causing arthritis (Peltier & Christian, 1959; Singer *et al.*, 1962; Williams & Kunkel, 1962; Carson *et al.*, 1978). The crucial difference between these conditions and rheumatoid arthritis appears to be that persistent local production of rheumatoid factor occurs in rheumatoid arthritic joints. Indeed there is a substantial body of indirect evidence suggesting that the interaction of rheumatoid factor with IgG in the joint may initiate inflammation (for review see Elson, Allen & Tite, 1980). How the chronic production of rheumatoid factors is maintained and what factors might favour their local production is unknown. We are currently testing some methods by which production of the anti-host allotypes might be induced locally in joints and examining the possibility that anti-host allotypes might exacerbate joint inflammation initiated by other means.

We should like to thank Dr J. Williams for her technical help. This work was supported by the Nuffield Foundation and the Medical Research Council.

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