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PARALLEL STUDIES ON THE EFFECT OF ANTI-LYMPHOCYTIC ANTIBODY ON CELL MEDIATED AND HUMORAL ANTIBODY RESPONSES IN THE RAT

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SUMMARY

The effect of prolonged treatment with anti-lymphocytic IgG raised in horses on cell mediated (homograft rejection) and humoral type immune responses has been investigated simultaneously in the same animal. The rejection of skin homografts precedes the development of circulating antibodies against alum precipitated bovine serum albumin but may follow the formation of agglutinating antibodies against sheep erythrocytes. High levels of antibodies against horse IgG are frequently detected prior to graft rejection.

INTRODUCTION

Recent reports from a number of laboratories have indicated that anti-lymphocytic antibody is capable of suppressing both cellular and humoral aspects of the immune response (James, 1967). However, apart from the work of Jeejeebuoy in thymectomized rats (1965, 1967) few attempts have been made to assess the effect of anti-lymphocytic antibody on both cellular and humoral responses in the same animal. We have, therefore, performed parallel investigations on the effect of antibody to rat lymphocytes on skin homograft survival and at the same time assessed its effect on their humoral response to alum precipitated bovine serum albumin, sheep erythrocytes and to the anti-lymphocytic antibody itself. Previous reports from this laboratory have already described the effect of the anti-rat lymphocyte antibody preparation used on the various antigens under test (Anderson, James & Woodruff, 1967; Clark, James & Woodruff, 1967; James & Anderson, 1967; James & Jubb, 1967).

MATERIALS AND METHODS

Details on the preparation and the properties of the horse anti-rat lymphocyte IgG preparation used in these studies have previously been reported (James & Anderson, 1967).

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These parallel studies on the effect of anti-lymphocytic IgG on both cell mediated and humoral antibody responses were performed in female rats of a hooded strain (weight range 185–255 g). The rats, twelve in number, received daily intraperitoneal injections of the anti-lymphocytic IgG preparation (1 ml of a 2 g/100 ml solution) for seven consecutive days prior to grafting (day -7 to -1). On day 0 skin grafting was performed by the method of Woodruff & Simpson (1955) the donor rats being an inbred Wistar strain. At the time of grafting all the animals were injected intraperitoneally with 5 mg of alum precipitated bovine serum albumin whilst six of the rats (see Fig. 2) also received an intravenous injection of 1×10^9 sheep erythrocytes. The rats continued to receive daily intraperitoneal injections of anti-lymphocytic IgG (0·5 ml of a 2 g/100 ml solution) from days 1 to 14 post grafting. The grafts were examined daily following the removal of the dressing on day 8 and serum samples were obtained at regular intervals for humoral antibody determinations.

The antibody to bovine serum albumin was determined by the Farr procedure (Farr, 1958) using a ^{131}I-labelled bovine serum albumin preparation containing 0·02 μg nitrogen per test. In many of the sera samples, less than 33% of the test antigen was bound and we were unable to estimate the antigen binding capacities. Because of this, the results have been expressed as a percentage of the test antigen bound by 0·5 ml of a 1:10 dilution of the serum. Further details of the procedure and the controls have been reported elsewhere (Pinckard, Weir & McBride, 1967; James & Jubb, 1967).

The production of anti-sheep erythrocyte agglutinins was assessed as previously described from this laboratory (James & Anderson, 1967). The results have been expressed as the reciprocal $\log_{10}$ of the titre.

The antibodies to normal horse IgG were estimated using tanned pyruvic aldehyde preserved sheep erythrocytes (Ling, 1961) sensitized with a 0·1 g/100 ml solution of normal horse IgG. Serial dilutions were performed using the Takatsky microtitrator. Each analysis included normal serum and standard antiserum controls as well as controls using unsensitized sheep erythrocytes. The controls using unsensitized cells indicated that the production of erythrocyte agglutinins in rats receiving sheep erythrocytes was so weak that it did not interfere with this test. The results are again expressed as the reciprocal $\log_{10}$ of the titre.

RESULTS

From Figs. 1 and 2 it can be seen that the anti-lymphocytic IgG treatment delayed the rejection of skin homografts in all but two animals thus confirming previous observations with this material (Anderson *et al.*, 1967). The rapid destruction of grafts observed in animals B and D (Fig. 2) can probably be attributed to a failure in the grafting procedure. It should perhaps be noted that the course of treatment used produced on average only a slight fall (less than 10%) in the peripheral blood lymphocyte count prior to grafting and injecting the test antigens.

As anticipated this prolonged course of anti-lymphocytic antibody treatment delayed and suppressed the primary immune response to bovine serum albumin and sheep erythrocytes. Although all the animals developed antibodies to bovine serum albumin the levels detected at 30–32 days were less than 10% those observed in animals receiving a much shorter course of anti-lymphocytic antibody treatment (James & Jubb, 1967). Of considerable interest is the time of appearance of antibodies to bovine serum albumin in relation to homograft rejection. In general, only low levels of antibody to bovine serum albumin were detected

at the time of complete rejection of the homograft (exception Fig. 1F) and these levels frequently showed a marked increase following the destruction of the graft.

Five out of six of the rats injected with sheep erythrocytes developed low levels of sheep erythrocyte agglutinins. This response was much weaker and later than that previously observed in control animals (James & Anderson, 1967). However, as in previous studies

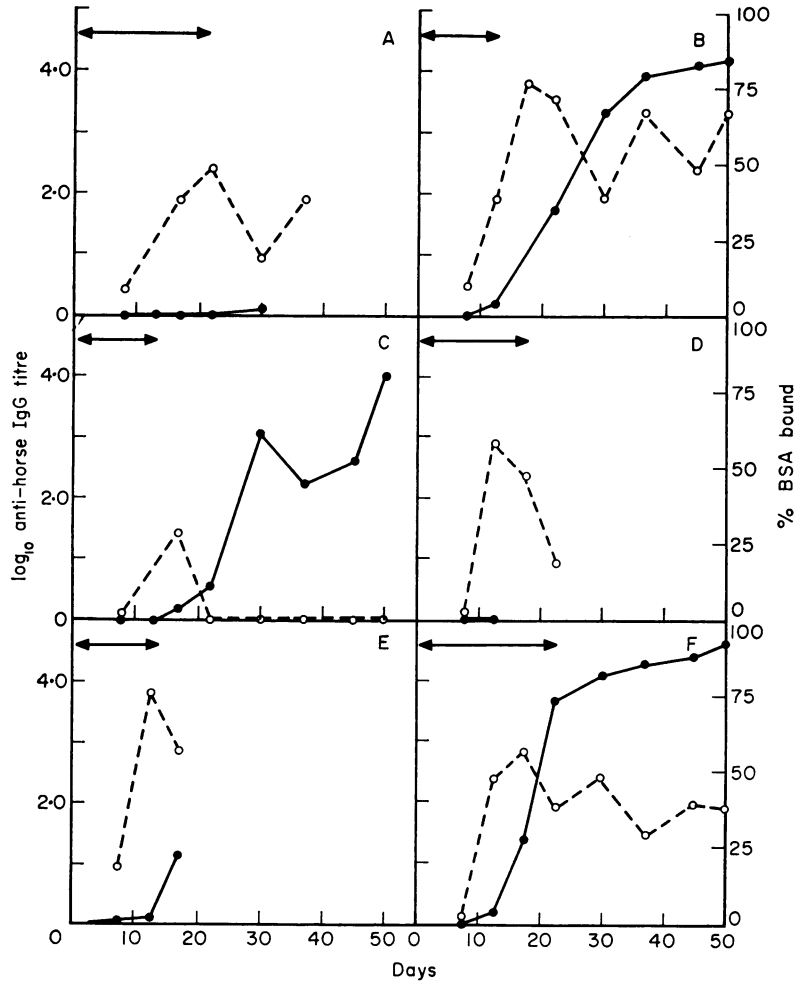

FIG. 1. The effect of anti-lymphocytic IgG on skin homograft survival and humoral antibody formation in hooded rats. Arrows represent graft survival; ●, Humoral antibody formation, antibodies to BSA; ○, humoral antibody formation, antibodies to horse IgG.

maximum erythrocyte agglutinin titres were observed before antibodies to bovine serum albumin could be detected and frequently prior to graft rejection.

All the rats developed antibodies against normal horse IgG. These were first detected about 14 days after commencing anti-lymphocytic IgG treatment and high levels were frequently observed prior to graft rejection. However, there was no apparent correlation

between the titre of antibodies to horse IgG and the time of graft rejection. The peak response usually coincided with the peak response to sheep erythrocytes.

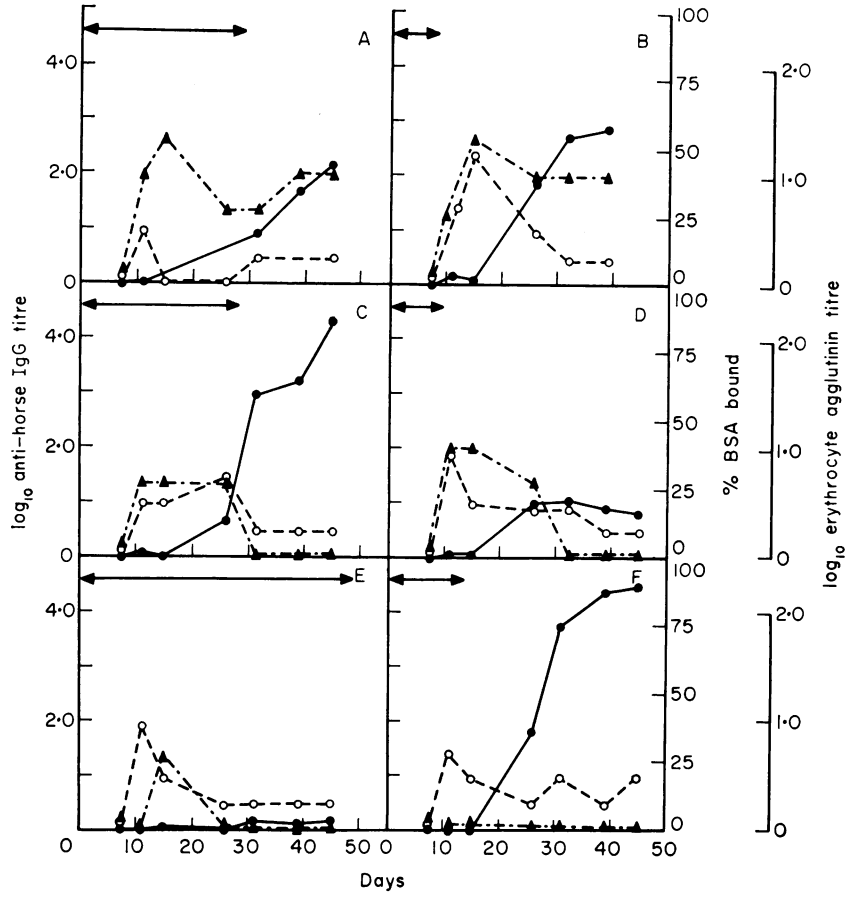

FIG. 2. The effect of anti-lymphocytic IgG on skin homograft survival and humoral antibody formation in hooded rats. Arrows represent graft survival; ●, Humoral antibody formation, antibodies to BSA; ○, humoral antibody formation, antibodies to horse IgG; ▲, humoral antibody formation, agglutinating antibodies to sheep erythrocytes.

DISCUSSION

It is appreciated that in studies such as these the intensity and course of the various immune responses might be influenced strongly by antigenic competition and that the apparent absence of humoral antibody might be a reflection of the insensitivity of the technique of detection or the combination of the antibody *in vivo* with circulating antigen. Nevertheless it is apparent that anti-lymphocytic antibody may readily inhibit both cell mediated and humoral antibody type immune phenomena in one and the same animal. These results do not support the recent suggestion of Levey & Medawar (1967) that

anti-lymphocytic antibody is less able to oppose immunization by mobile antigens which are able to travel directly to the spleen, than to oppose immunization mediated through peripheral mechanisms. The relative effect is, as one might expect, dependent upon the antigen under test.

The results also indicate that even prolonged treatment with anti-lymphocytic antibody does not prevent humoral antibody formation. Thus as in the case of homografts, animals eventually recover their capacity to produce humoral antibodies, presumably due to the subsequent development of immunologically competent cells or to the functional recovery of pre-existing lymphocytes (James & Jubb, 1967). Of interest, however, is the rate at which the animal recovers its capacity to produce antibodies against the various antigens or to reject the homograft. Peak levels of antibodies to sheep erythrocytes (and horse IgG) and the rejection of skin homografts are apparent before the development of significant levels of antibodies to bovine serum albumin. This, however, may only be a reflection of basic differences in the normal immune response to these antigens and not to preferential recovery of specific immunological potential.

The inability of anti-lymphocytic antibody to completely inhibit humoral antibody formation against itself confirms previous observations in rats (Currey & Ziff, 1966; Clark *et al.*, 1967). However, it still remains to be established whether or not this inability directly affects the immunosuppressive efficiency and therapeutic potential of these materials.

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