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Disappearance of IgG2B autoantibodies associated with recovery from anaemia

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SUMMARY

Mice immunized with cross-reacting rat erythrocytes develop autoimmune haemolytic anaemia as indicated by haematological data and erythrocyte autoantibodies. With continued immunization the mice recover haematologically but remain Coombs' positive. Coombs' tests were performed using monospecific antisera to determine whether the recovery from anaemia was associated with a change in the class or subclass of the autoantibodies produced. In both splenectomized mice and in unsplenectomized mice the following subclasses of erythrocyte autoantibodies were present: IgG1, IgG2A, IgG2B. IgA autoantibodies were not detected and IgM autoantibodies were only detected in splenectomized mice 1–3 weeks after the IgG autoantibodies had appeared. After six immunizations the frequency of IgG2B autoantibodies decreased and by the tenth immunization and thereon, IgG2B autoantibodies were not detected. It is proposed from these results that the anaemia is caused by IgG2B autoantibodies and that the sudden exacerbation in the anaemia that occurs in splenectomized mice is due to the production of IgM autoantibodies.

INTRODUCTION

Mice of some strains immunized i.p. with antigenically cross-reacting rat erythrocytes develop a disease resembling warm-type autoimmune haemolytic anaemia of man (Cox & Keast, 1973; 1974b). Susceptible mice show evidence of anaemia, reticulocytosis, shortened survival of ^{51}Cr-labelled syngeneic erythrocytes *in vivo*, and a high proportion are positive in direct Coombs' tests. Splenectomized mice become more anaemic and remove syngeneic erythrocytes from circulation faster than unsplenectomized mice. However, the disease is not progressive even in mice immunized at weekly intervals for 12 weeks. With continued weekly immunization the blood pictures of the mice revert to near normal levels by the 12th week except for the erythrocyte autoantibodies which persist very strongly throughout (Cox & Keast, 1974b). Since the various classes and subclasses of immunoglobulins vary in their biological functions (Speigelberg, 1974) we undertook to investigate whether the recovery phase of this autoimmune process was associated with a change in the class or subclass of immunoglobulins that make up the erythrocyte autoantibodies. In addition we investigated whether the classes or subclasses of erythrocyte autoantibodies in the more anaemic splenectomized mice were different from those in the unsplenectomized mice. Our results show that IgG2B autoantibodies were present in most mice during the most severe phase of the anaemia but were completely absent late in the recovery phase. Also IgM autoantibodies were only detected in the splenectomized mice.

MATERIALS AND METHODS

Mice and rats. Inbred C_3H mice of the same strain as those used for the initial studies of this mouse model of autoimmune haemolytic anaemia (Cox & Keast, 1973) were purchased from the Institute of Medical and Veterinary Science in Adelaide. Inbred WAG rats were kindly given to us by Dr Keast, Department of Microbiology, University of Western Australia.

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Immunization protocol. Mice aged 7–11 weeks were immunized i.p. at weekly intervals with 2×10^8 erythrocytes from inbred WAG rats as previously described (Cox & Keast, 1974b).

Methods for splenectomy and haematological studies. These are given in other publications (Cox & Keast, 1974a, 1974b).

Antiglobulin (Coombs') tests. The method for Coombs' tests has been described (Cox & Keast, 1973). The rabbit antiserum against mouse immunoglobulins used for the earlier studies (Cox & Keast, 1973) was also used in these studies. In addition rabbit antisera monospecific for the following mouse immunoglobulins: IgM, IgA, IgG1, IgG2A, IgG2B, were obtained from Meloy Laboratories Incorporated. Coombs' tests were performed using two dilutions of each of these monospecific antisera: 1/500 and 1/1500. These dilutions were shown to give optimal reactions against various erythrocytes ranging from weakly Coombs' positive to strongly Coombs' positive as determined using the broad-spectrum Coombs' serum.

RESULTS

The pattern of anaemia and reticulocytosis that occurred in mice immunized at weekly intervals with rat erythrocytes for 6 weeks, or for 12 weeks, is shown in Fig. 1. These results are in accord with those

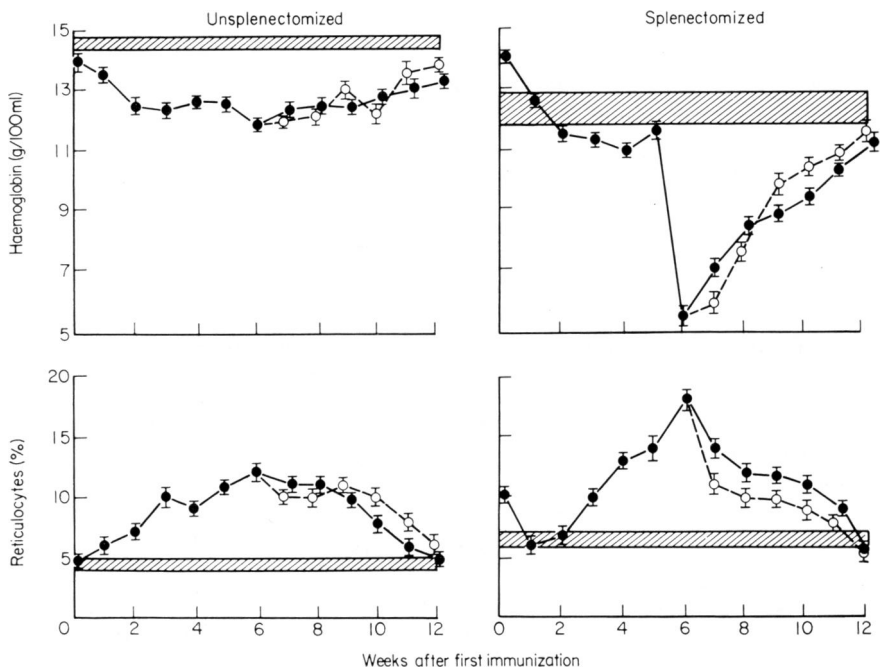

FIG. 1. Haemoglobin concentrations and reticulocyte levels in C_3H mice immunized at weekly intervals with rat erythrocytes. The shaded areas represent ±1 s.d. from the mean for unimmunized mice. The points are means and the bars are the limits of ±1 s.d. from the mean. (○) Immunized each week; (●) immunized each week up to week 6. The minimum number of mice at any of the points is seven.

published previously (Cox & Keast, 1974b). The main observations are that splenectomized mice became more anaemic than unsplenectomized mice; the peak anaemia occurred around week 6 and the mice had almost totally recovered by week 12 irrespective of whether immunization was continued at weekly intervals after week 6.

The results of direct Coombs' tests are shown in Fig. 2. By the 5th week of immunization all mice were Coombs' positive with the broadspectrum Coombs' serum. Autoantibodies of the IgA subclass were not detected. IgG1 and IgG2A autoantibodies were present in a high proportion of the mice and persisted throughout the recovery phase. In contrast IgG2B autoantibodies were present in most mice by week 6 but gradually disappeared and were absent by week 10. IgM autoantibodies were not found in unsplenectomized mice, but, surprisingly, were detected about 2 weeks after the IgG antibodies in almost half of the splenectomized mice and were present for 1–2 weeks.

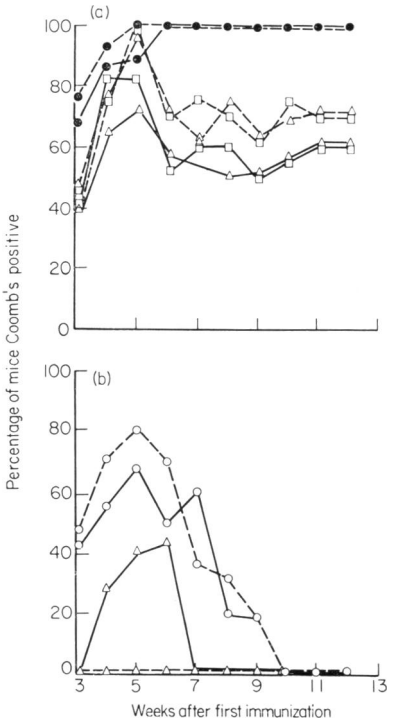

FIG. 2. The incidence of positive direct Coombs' tests in mice immunized at weekly intervals for twelve weeks with rat erythrocytes. The number of mice positive in direct Coombs' tests is shown as a percentage of the total number of mice tested. The minimum number of mice tested on any week was 12. Splenectomized mice (———); unsplenectomized mice (- - - -). (a) (●) Broad spectrum Coombs' serum; (□) specific anti-IgG1 Coombs' serum; (△) specific anti-IgG2A Coombs' serum. (b) (○) Specific anti-IgG2B Coombs' serum; (△) specific anti-IgM Coombs' serum.

DISCUSSION

Mice immunized at weekly intervals with rat erythrocytes develop autoimmune haemolytic anaemia from which they recover except for the persistence of erythrocyte autoantibodies (Cox & Keast, 1974b). The results from the present study are consistent with this report. In this study it was undertaken to characterize the classes and subclasses of erythrocyte autoantibodies in both splenectomized mice and unsplenectomized mice at various stages of the disease process to determine whether changes in the nature of the erythrocyte antibodies were associated with recovery from the anaemia.

The presence of IgG1 and IgG2A autoantibodies in a high proportion of mice throughout the anaemic phase and the recovery phase, suggests that these autoantibodies are not involved in the premature destruction of the erythrocytes. The observation that IgG2B autoantibodies were present in most mice during the anaemic phase and their gradual disappearance was associated with the recovery of haemoglobin levels suggests that these antibodies may have a prime role in the destruction of the erythrocytes. In the mouse, IgG2B antibodies have been implicated with complement-mediated cell lysis (Nussenzweig, Merryman & Benacerraf, 1964). Thus it would be appropriate in this experimental model to measure *in vitro* phagocytosis and lysis rates of erythrocytes that are carrying various subclasses of autoantibodies.

The appearance of IgM autoantibodies correlated well with a sudden exacerbation of the anaemia. IgM antibodies are known to be very efficient in complement-mediated lysis and in facilitating phagocytosis (Speigelberg, 1974). Thus the previous observation that splenectomized mice became more anaemic and cleared ^{51}Cr-labelled erythrocytes from circulation faster than unsplenectomized mice

(Cox & Keast, 1974b) may be due to the IgM erythrocyte autoantibodies in splenectomized mice.

The absence of IgGA autoantibodies is consistent with the rarity of these erythrocyte autoantibodies in man (Dacie, 1968). There are several explanations for the disappearance of IgG2B autoantibodies. Repeated immunization with rat erythrocytes may have lead to clonal senescence (Williamson & Askonas, 1972) of IgG2B autoantibody-producing clones. Against this explanation is the observation that in the New Zealand Black mouse IgG2B autoantibody-producing cells have persisted together with IgM, IgA, IgG1 and IgG2A autoantibodies, in mice aged 6, 9 and 12 months (DeHeer & Edgington, 1974). In other studies C_3H mice responding to BALB/c mouse spleen cells have been shown to shift from production of IgG2 antibodies to IgG1 antibodies (Harris & Harris, 1975). However, whether these shifts in production of classes of antibodies are a sign of some immunoregulatory system remains to be elucidated. It is clearly possible that the mouse may be able to utilize some control mechanism to turn off production of deleterious autoantibodies.

The reasons for the appearance of IgM autoantibodies after the detection of IgG autoantibodies, and only in splenectomized mice, is not readily apparent. It could be argued that entirely new clones of auto-aggressive cells were generated (Cunningham, 1974) following two or three immunizations with rat erythrocytes. Alternatively some antigen on the rat erythrocytes that cross reacts with the mouse erythrocytes may have been processed less efficiently in the splenectomized mice but because of continual presentation, eventually reached a strongly immunogenic dose.

From this study it is proposed that the symptoms of autoimmune haemolytic anaemia that follow in mice immunized with rat erythrocytes are due to the production of IgG2B erythrocyte autoantibodies and that the sudden exacerbation in the anaemia that occurs in the splenectomized mice is due to the production of IgM autoantibodies.

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