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Relative Avidity of Antibodies towards Sheep Red Blood Cells in New Zealand Black Mice

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Summary. Antibody-mediated inhibition and intercellular haemolysin transfer were studied in New Zealand Black (NZB) mice. The results, which support the hypothesis of the production of a low avidity IgG antibody, are discussed in relation to a disturbed thymic function.

INTRODUCTION

Mice of the New Zealand Black (NZB) strain exhibit a prolonged primary and secondary IgM and IgG plaque-forming cell (PFC) response towards sheep red blood cells (SRBC). This finding is already present before the autoimmune state becomes overt. It was suggested that a disturbed feed-back mechanism of the immune response, caused by the formation of a low avidity IgG antibody, may play a role in the increased antibody response (Elkerbout and Hijmans, 1974). Background PFC values, which can be considered to be caused by enteric bacteria (Cheng and Trentin, 1967) are also increased in NZB mice (Baum, 1969; Elkerbout and Hijmans, 1974). This observation, together with the increased IgM serum levels (Warner and Wistar, 1968) could support the hypothesis of a deficient control of the synthesis of antibodies. This possibility was analysed in the present study by investigating the influence of the administration of immune sera, derived from a normal mouse strain, on antibody formation in NZB mice.

A further step was the study in immunized NZB and BALB/c mice of intercellular haemolysin transfer, which is inversely related to avidity (Taliaferro, Taliaferro and Pizzi, 1959). Our data indicate that a defect of the qualitative development of antibody synthesis may result in a quantitative effect in NZB mice.

MATERIALS AND METHODS

Inhibition studies

The majority of the materials and methods have already been described in detail in the preceding article. These will only be mentioned in summary form here.

Animals. NZB and BALB/c mice maintained and bred in our own colony were used.

Antigen challenge. A standard dose of 4×10^8 SRBC was injected in the tail vein in a volume of 0·2 ml. The SRBC had been washed three times in phosphate-buffered saline (PBS).

Preparation of antiserum. Nine-month-old BALB/c mice were bled 11 days after one course of SRBC.

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Recipients. Nine-month-old NZB mice received 2 ml of antiserum intraperitoneally. The antiserum was administered 8 days after immunization with SRBC.

Plaque formation. On various days after the administration of the antiserum the PFC response in NZB mice was determined. Data from the preceding study of the PFC response in NZB and BALB/c mice, treated with 4×10^8 SRBC only, served as controls.

Serum haemolysin antibody determination. Using a microtitration method serum haemolysins were determined in immunized NZB and BALB/c mice and in immunized NZB mice treated with antiserum. On various days after immunization blood was collected from the tail or from the axillary vessels. The wells in the titration plates contained 0·025 ml PBS, 0·025 ml 2 per cent SRBC, 0·025 ml 10 per cent guinea-pig complement and 0·025 ml serum. Titration was performed by 2-fold dilution series. Antibody activity is given as 100 per cent haemolysis after incubation at 37° for 1 hr. IgG antibody was determined by its 2-mercaptoethanol (2-ME) resistance, added before titration. The sera were diluted with equal volumes of 0·15 M 2-ME in PBS and incubated at 37° for 3 hr. The remaining haemolysin activity was then determined.

Transfer studies

Taliaferro, Taliaferro and Pizzi (1959) described a method of titrating intercellular haemolysin transfer by combining the ^{51}Cr red cell system with haemolysin titration. In this test sensitized SRBC are mixed with ^{51}Cr-labelled unsensitized cells. If transfer of haemolytic antibodies occurs, this will result in the sensitization of the isotope-labelled cells. This transfer can be detected and quantified by adding complement to the system and then measuring the radioactivity of the supernatant. It is clear that an inverse relationship between the degree of avidity and the degree of transfer, and thus in the actual test the radioactivity of the supernatant, can be expected. In general the original method was followed in detail.

Antiserum was derived from NZB and BALB/c mice on various days after immunization with 4×10^8 SRBC.

Complement. Guinea-pig serum was absorbed with washed SRBC. After absorption the complement was titrated with a standardized haemolysin.

Haemolysin titration. The sera were titrated in 50 per cent units per ml with 12 units of complement.

^{51}Cr-labelled SRBC. 10 ml fresh, defibrinated sheep blood was mixed with 100 μCi ^{51}Cr for 2 hr. Then the cells were washed five times. Before using in the transfer test the labelled cells were standardized spectrophotometrically and with a gamma counter.

Transfer test. Standardized SRBC suspensions were mixed with different amounts of 50 per cent units of a given serum. Tubes containing SRBC and different serum dilutions were kept at room temperature for 30 min. After centrifugation the packed cells were resuspended. Standardized ^{51}Cr-labelled cells were added and the mixture of cells was kept at 37° for 30 min. After 30 min haemolysis was arrested by adding 2 volumes of sodium citrate followed by centrifugation. A gamma count of the liberated haemoglobulin gave a direct measure of the haemolysin which had been transferred from the sensitized SRBC to the ^{51}Cr-labelled SRBC. The final results were expressed in percentage transfer. Percentage of transfer is the reciprocal value of the number of 50 per cent units of a given haemolysin which needs to be absorbed on carrier SRBC to cause 50 per cent haemolysis of unsensitized ^{51}Cr-labelled SRBC.

RESULTS

PFC RESPONSE IN NZB MICE TREATED WITH ANTISERUM

From Figs 1 and 2 it can be seen that IgM and IgG antibody response was lower in the immunized NZB mice treated with antiserum than in the NZB mice only treated with SRBC. Besides, the plaques looked convincingly smaller in the antiserum treated NZB mice than in the control groups. A group, consisting of five NZB mice was injected with serum derived from unimmunized BALB/c mice. Fifteen days after immunization these animals showed a PFC response comparable with the NZB mice, who had not received antiserum.

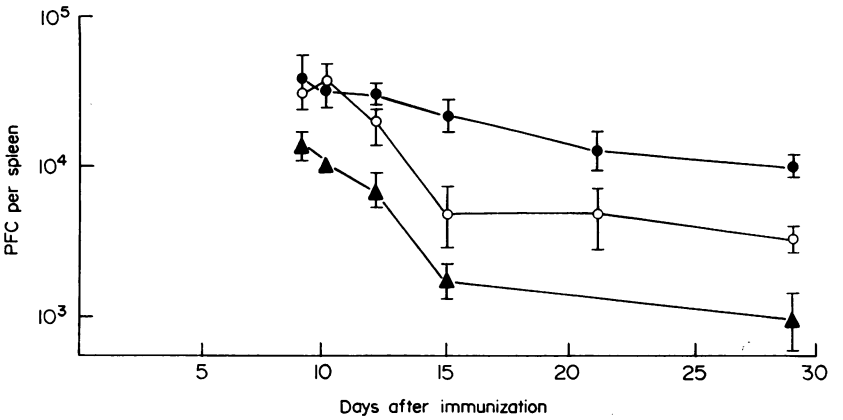

FIG. 1. IgM PFC response in 9-month-old NZB and BALB/c mice immunized with 4×10^8 SRBC: (○) immunized and antiserum-treated NZB mice; (●) immunized NZB; (▲) immunized BALB/c mice. The values shown are the logarithmic means ± 1 s.e.

HAEMOLYSIN TITRES

From Fig. 3 it can be seen that comparing total antibody titre of a group of 9-month-old antiserum-treated NZB mice, containing five animals, with two untreated groups, consisting of five NZB and five BALB/c mice, the titres of the antiserum-treated NZB mice approximate the values in BALB/c mice, 12, 15 and 21 days after immunization. Mercaptoethanol-resistant titres were found to be lower in the antiserum-treated NZB mice than in BALB/c mice, 12, 15 and 21 days after immunization (Fig. 4).

In summary it can be said that in NZB mice lower PFC responses and haemolysin titres exist after administration of immune serum derived from SRBC-treated BALB/c mice. These antisera had been obtained in the early IgG period.

TRANSFER TEST

Transfer tests of pooled sera were performed on various days after immunization. Initially the titre as well as the avidity was higher in NZB mice than in BALB/c mice. The relative increase of the titres in NZB mice persisted, if compared with BALB/c mice, throughout the observation period of 29 days (Table 1). The figures of the transfer test indicate that the avidity of antibodies in NZB animals was lower than in the BALB/c mice during the second half of the test period.

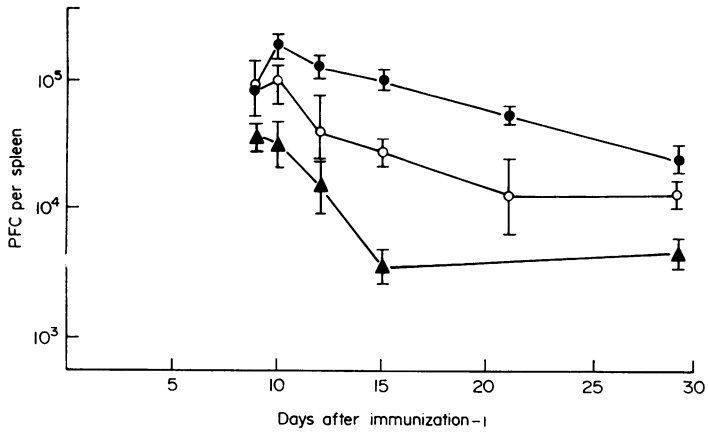

FIG. 2. IgG PFC response in 9-month-old NZB and BALB/c mice immunized with 4×10^8 SRBC: (○) immunized and antiserum-treated NZB mice; (●) immunized NZB; (▲) immunized BALB/c mice. The values shown are the logarithmic means ± 1 s.e.

DISCUSSION

In studies on the avidity of antibodies to SRBC in NZB mice by the administration of immune serum and the intercellular transfer of haemolysin, we found a normalizing effect on the PFC response and haemolysin titres. If antibody acts by combining with antigenic determinants then it is reasonable to believe that in NZB mice these determinants are still available 8 days after immunization, this being the interval between immunization and antibody administration. Using normal mouse strains Henry and Jerne (1968) demonstrated that suppression was most pronounced if antibody was given before the SRBC. When antibody was administered 2 days after immunization no suppression occurred. The

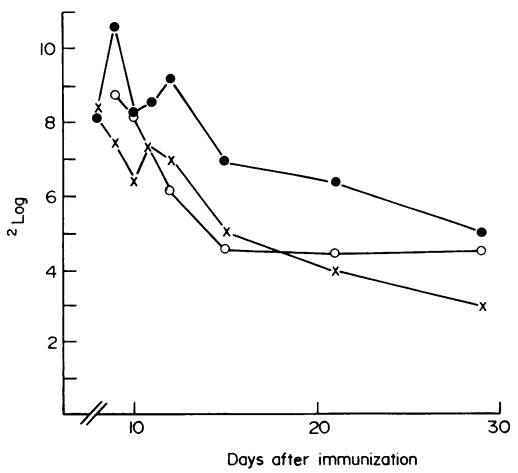

FIG. 3. Total serum haemolysin titre in 9-month-old NZB and BALB/c mice immunized with 4×10^8 SRBC: (○) immunized and antiserum-treated NZB mice; (●) immunized NZB; (×) immunized BALB/c.

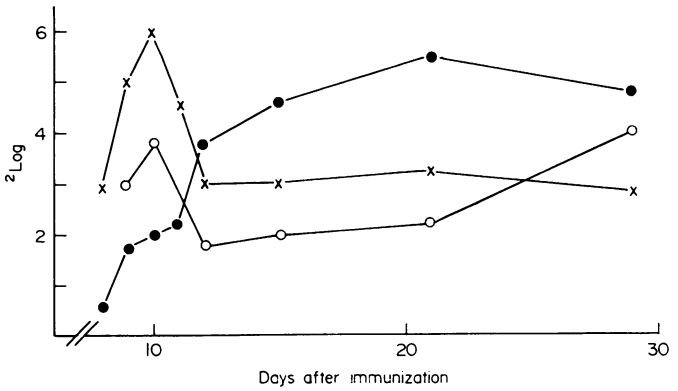

FIG. 4. Mercaptoethanol-resistant haemolysin titre in 9-month-old NZB and BALB/c mice immunized with 4×10^8 SRBC: (○) immunized and antiserum-treated NZB; (●) immunized NZB; (×) immunized BALB/c.

immune suppression in NZB mice caused by late immune serum administration supports the hypothesis of an IgG antibody of relatively low avidity in these animals. Antibody-mediated inhibition studies in these mice have also been presented by Morton and Siegel (1972) and Röllinghoff and Warner (1973). Both these studies appeared to negate the possibility of a deficiency in the IgG antibody-mediated feed-back mechanism. In the study of Morton and Siegel (1972) no suppression occurred with antiserum derived from NZB mice which was obtained during the early primary response (4 and 7 days after immunization). This could be explained by the fact that in our study IgG PFC are first demonstrable 6 days after immunization and that the 2-ME-resistant antibody titre is negative in most NZB mice tested (nineteen out of twenty-four) 8 days after immunization. Hyperimmune NZB serum turned out to be immunosuppressive just as the NZB serum, used by Röllinghoff and Warner (1973) which was obtained 14 days after immunization. One can assume that both these NZB sera had high IgG titres. If administered prior to SRBC Henry and Jerne (1968) demonstrated that in normal mice even small concentrations of IgG antibody cause a marked suppression of the primary response. As the NZB antisera were given 24 hr before and at the same time as the immunization one can imagine that on account of the high IgG titre a failure in the feed-back mechanism could not be detected.

TABLE 1

TRANSFER TEST IN 9-MONTH-OLD NZB MICE AND BALB/c MICE IMMUNIZED WITH 4×10^8 SRBC

Days after immunization	Titre in 50% units per ml		Percentage transfer	
	BALB/c	NZB	BALB/c	NZB
7	1930	2340	23	17·5
8	1210	3020	25	15
12	200	1060	24	11·5
15	83	1000	12	13
21	25	208	9	20
29	50	200	7	10

In the transfer test avidity of IgM and IgG antibodies are measured. Initially higher avidity values were found in the NZB strain. As can be seen from Figs 3 and 4 the avidity of antibodies in the NZB mice will be largely determined by 2-ME-sensitive antibodies up to 12 days after immunization. Thereafter 2-ME-resistant antibodies predominated in the NZB and in the BALB/c mice. Hence the avidity values in the two strains can be compared more easily in this period. During this period with IgG predominance antibody avidity was lower in NZB mice. From Fig. 4 it can also be seen that control BALB/c strain showed a peak 2-ME-resistant antibody titre 10 days after immunization. Considering total haemolysin titre (Fig. 3) 2-ME-sensitive antibody titre was very low at this moment. After this 'switch' from 2-ME-sensitive to 2-ME-resistant antibody avidity started to rise. In NZB mice such a sudden 'switch' did not occur. These results can support the idea of the presence in NZB mice of an IgG antibody against SRBC of relatively low avidity. This antibody is not able to suppress the IgM response resulting in a lack of a 'switch' and a prolonged antibody response.

Taliaferro *et al.* (1959) described an inverse relationship between haemolysin titres and the percentage of transfer. Based on these data one can assume, even if similar values of transfer in NZB and BALB/c mice have been found, that avidity of these NZB antibodies is relatively low. As a matter of fact the haemolysin titres in NZB were considerably higher than in BALB/c mice during the observation period of several weeks. On the other hand Petty, Steward and Soothill (1972) did not find a clear relationship between affinity and amount of antibody and they underlined the strain specificity of this association. Affinity studies were also performed by Petty and Steward (1972) in NZB mice, using human transferrin as antigen. These authors observed that the relative affinity of antibodies produced in NZB mice was lower than in other strains tested. In the same laboratory (Alpers, Steward and Soothill, 1972) it was shown that mice producing high affinity antibody eliminate antigen more effectively than those producing low affinity antibody. The immunopathological significance of this phenomenon in NZB mice has been discussed by Petty and Steward (1972). It was suggested that the antibodies produced to the antigen involved in the pathogenesis of the soluble complex disease of NZB mice may also be of low affinity, which results in failure of antigen elimination.

The next question is if the production of low avidity antibodies is related to the functioning of the thymus. Morphological abnormalities of the thymus in NZB mice have been published by Burnet and Holmes (1964) and de Vries and Hijmans (1966), and an impaired thymus-dependent cell-mediated immunity has been described by others (Stutman, Yunis and Good, 1968; Cantor, Asofsky and Talal, 1970; Leventhal and Talal, 1970). Finally, Stutman (1972) reported a deficit of thymus-dependent lymphocytes (T cells) in NZB mice. Allison, Denman and Barnes (1971) suggested that T cells not only co-operate with (non-thymus-derived) B cells but also exert a specific feed-back control on the synthesis of antibodies by B cells. These authors distinguish feed-back mechanisms as: (1) T cells controlling B cells; and (2) IgG antibody produced by B cells, which inhibit helper T cells. These considerations are based on the knowledge that the SRBC antibody response is thymus-dependent. If it could be shown that the response to thymic-independent antigens in NZB mice is not different from the response in control strains, our speculations on the central role of the thymus in the pathogenesis of autoimmune disease would gain strength. This in itself does not exclude a virus aetiology of these diseases (Allison *et al.*, 1971), nor does it diminish the significance of additional factors, such as disturbance of the macrophage function (Thomas and Weir, 1972). If it could be proven definitely that

a thymic deficiency is responsible for the autoimmune phenomena in the NZB animals, attempts to restore its function by transplantation or via the administration of thymus factors would be justified.

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