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Idiotype Suppression in Immune Response of BALB/c Mice to α-1,3-Dextran by Antiidiotypic Antibody (40567)[1]

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There are three plasmacytoma immunoglobulins (MOPC-104E, J558, and UPC-102) with antibody activity to α-1,3-dextran (Fraction S from Leukonostoc mesenteroides NRRL B-1355) (1–4). These three idiotypes have also been detected in the serum of BALB/c mice immunized with this dextran (5–7).

Idiotype suppression in mice by the injection of xenogeneic or allogeneic antiidiotypic antibody prior to the antigen is a known phenomenon (8–15). Since the two dextran-specific idiotypes of MOPC 104E and J558 myeloma proteins differ from each other by only three or four amino acids at the VH-7 region (1, 16), it appeared to be of interest to determine the effect of antibody to these idiotypes on the dextran response of BALB/c mice. In experiments performed by Weiler *et al.*, the immune response to dextran and the expression of the J558 idiotype were suppressed in the F_1 progeny of SJL females and BALB/c males, when the SJL females received BALB/c spleen cells secreting antidextran antibodies, were then immunized 18 days later with J558 myeloma protein and mated to BALB/c males (17). The results of the presently reported experiments indicate that idiotype suppression can also be achieved in a syngeneic system of adult BALB/c mice. In these experiments, these mice were injected with either MOPC-104E IgM or J558 IgA, and subsequent immunization of these animals with dextran resulted in antibody in which the idiotype, either J558 or MOPC-104E, that had been used for the prior injection was suppressed.

Materials and methods. α-1,3-Dextran was a gift from Drs. Allen Jeans and F. R. Seymour, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois. The *E. coli* lipopolysaccharide (LPS) was purchased from DIFCO Laboratories (Detroit, Mich.). BALB/c mice were obtained from the Cancer Research Center, Frederick, Maryland. MOPC-104E and J558 tumors were originally supplied by Dr. Michael Potter (National Cancer Institute, Bethesda, Md.) and were maintained in our laboratory by serial subcutaneous transfer of plasmacytoma cells in BALB/c mice.

Isolation of myeloma proteins and preparation of antiidiotypic antisera in rabbits and BALB/c mice. MOPC-104E IgM and J558 IgA were isolated from the sera of the tumor-bearing mice by affinity chromatography using α-1,3-dextran-conjugated Sepharose 4-B columns as described by Hiramoto *et al.* (18). The purity of the so-prepared immunoglobulins was tested by immunoelectrophoresis and passive hemagglutination of erythrocytes coated with the respective antiidiotypic antibody.

Rabbit antisera to myeloma proteins J558 IgA and MOPC-104E IgM were prepared according to established technics (19). The sera were decomplemented and absorbed extensively with glutaraldehyde-treated normal mouse serum and normal mouse immunoglobulins to render them antiidiotypic. The anti-104E serum was passed through a J558 IgA-conjugated Sepharose 4-B column and anti-J558 antiserum through a MOPC 104E IgM column. The specificity of the antisera was tested by passive hemagglutination, hemagglutination inhibition, and gel diffusion with 4% polyethylene glycol incorporated in the agar (20). The IgG fractions of the rabbit antisera were isolated by DEAE-Sepharose chromatography and absorbed with sheep erythrocytes and normal mouse erythrocytes.

Antiidiotypic antibody to J558 IgA or MOPC-104E IgM was also raised in BALB/c mice according to the method described by Yakulis *et al.* (21). After evidence

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for the presence of antibody was obtained by passive hemagglutination and hemagglutination inhibition the mice were challenged with 8 μg of α-1,3-dextran (22). The controls for the dextran response consisted of normal BALB/c mice and BALB/c mice injected with only Freund's complete adjuvant. The immune response to sheep red blood cells (SRBC) and LPS was also tested in these four groups of mice.

Measurement of the immune response to dextran, SRBC, and LPS. (a) Coating of sheep erythrocytes. Sheep erythrocytes were coated with the IgG fraction of rabbit-anti-104E-IgM or anti-J558-IgA by chromic chloride according to the method of Gold and Fudenberg (23) with some modifications (22). α-1,3-Dextran was first conjugated to BSA (24) and then was coated to sheep erythrocytes with chromic chloride (22). LPS was coated to SRBC by the method described by Britton (25). Suspensions, 1%, of these coated SRBC were preapred.

(b) Quantification of anti-dextran plaque-forming cells. The mice were killed 5 days after antigen injection by cervical dislocation and the spleens were removed. A 1% suspension of spleen cells was prepared after washing the cells twice with RPMI medium (Grand Island Biological Co., Grand Island, N.Y.). Dextran-coated SRBC (0.2 ml) and 0.1 ml of splenic cells were added to 1 ml of 1% sea plaque agar (Marine Colloid, Inc., Rock Island, Me.) solution. This mixture was layered on a bed of 4 ml of 2% sea plaque agar in a 60 × 15-mm petri dish (Falcon Plastic, Oxnard, Calif.). The agar was allowed to gel at room temperature then the petri dishes were placed in an incubator at 37° for 1 hr. Monospecific rabbit anti-mouse IgM in a dilution of 1:200 (1 ml/plate) was added along with 2 ml of guinea pig complement (1:200). The petri dishes were placed back in the incubator for 2 hr. All preparations were made in triplicate. The plaques were counted at 4× magnification. In order to test the specificity of antidextran plaques, the plaque inhibition experiments were attempted with rabbit antiidiotypic antiserum to MOPC 104E IgM (1:150) and J558 IgA (1:150). The above antisera, 100 μl, was added to the mixture of coated SRBC, spleen cells, and 1% agar and this mixture was layered and the remaining steps were followed as described above.

(c) Quantification of 104E and J558 idiotype-secreting cells. The reverse hemolytic plaque assay described by Molinaro and Dray was adapted to the needs of this study (26). Sheep erythrocytes were coated with the IgG fraction of rabbit antiidiotypic antisera to 104E IgM or to J558 IgA, and mixed with spleen cells and 1% agar solution as described above. The plates were incubated for 1 hr at 37°. Then, for the demonstration of the 104E or J558 idiotype-secreting cells, 1 ml of the respective diluted antiidiotypic antiserum (1:200 for 104E IgM and 1:150 for J558) was added. The petri dishes were incubated for an additional hour. Then 2 ml of guinea pig complement (1:200) was added after washing the antisera of the plates which then were incubated for 1 h more. Inhibition of these idiotypic plaques by α-1,3-dextran was attempted. This antigen, 1 to 50 μg, was added to the mixtures of spleen and target cells incubated for 1 hr prior to the addition of the antisera and complement. The plaques were counted as above. The immune response to SRBC was measured by the Jerne hemolytic plaque assay (27) and the anti-LPS response by the hemolytic plaque assay as described by Britton (25).

The number of plaques/whole spleen was transformed to its $\log_{10}$ and statistically analyzed by Student's *t* test. The means that are recorded in Tables I and II represent the antilogs of the means of the logarithmically transformed data (28).

Results. Production of antiidiotypic antisera to J558 and MOPC-104E myeloma proteins in BALB/c mice. Normal BALB/c mice (6 weeks old) were injected weekly with MOPC-104E IgM or J558 IgA in complete Freund's adjuvant. Antiidiotypic antibodies were demonstrated 4–6 weeks later. The passive hemagglutination titers were usually in the range of 1:320 to 1:640. The antiidiotypic antisera reacted only with the specific myeloma protein, against which the antiserum was raised. They failed to react with normal mouse IgG, normal BALB/c serum, and with myeloma proteins from LPC-1, MOPC-300, and MOPC-315 in the hemagglutination and gel diffusion tests. Anti-J558 IgA did not react with MOPC-104E IgM and vice versa.

TABLE I. THE ANTIDEXTRAN RESPONSE AND THE IDIOTYPIC NATURE OF ANTIDEXTRAN ANTIBODIES IN BALB/C MICE PREIMMUNIZED WITH MYELOMA PROTEINS MOPC 104E OR J558

Groups of mice	Antidextran Ab-secreting cells	104E Id-secreting cells	J558 Id-secreting cells
Control	5.09 ± 0.04*	5.22 ± 0.02	4.85 ± 0.10
	123,026	165,958	70,794
Adjuvant injected	5.00 ± 0.07	5.20 ± 0.03	4.85 ± 0.07
	100,000	158,489	70,794
MOPC 104E IgM injected	5.11 ± 0.04	2.02 ± 0.47[b]	4.91 ± 0.03
	128,824	105	81,283
J558 IgA injected	4.80 ± 0.07[a]	5.10 ± 0.03[c]	3.08 ± 0.29[d]
	63,095	125,892	1202

* Mean of $\log_{10}$ of the number of PFC/spleen ± SE. The recorded number of plaque-forming cells/spleen is the antilog of the mean for 16–18 mice. [a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.01$, [d]$P < 0.0001$ (all compared with control).

TABLE II. IMMUNE RESPONSE TO SRBC AND *E. coli* OF BALB/C MICE IMMUNIZED WITH MYELOMA PROTEINS 104E IGM OR J558 IGA

Group of mice	Anti-SRBC plaques	Anti-LPS plaques
Control	5.3 ± 0.02*	4.7 ± 0.008
	199,526	50,118
Adjuvant injected	5.2 ± 0.02[a]	4.6 ± 0.84
	158,489	39,810
MOPC 104E IgM injected	5.1 ± 0.02[b]	4.9 ± 0.04[c]
	125,892	79,432
J558 IgA injected	5.2 ± 0.06[a]	4.7 ± 0.02
	158,489	50,118

* Mean of $\log_{10}$ PFC/spleen ± SE. The recorded number of plaque-forming cells represents antilog of the mean for six or more mice. [a]$P < 0.04$, [b]$P < 0.05$, [c]$P < 0.01$ (all compared with control).

The hemagglutination of J558 IgA-coated mouse erythrocytes with anti-J558 IgA was inhibited by prior incubation of the coated erythrocytes with dextran. The coated mouse red cells were also incubated with antiidiotypic antiserum that had been previously absorbed with serum from J558 tumor-bearing mice, 5 μg of isolated J558 IgA, or serum from BALB/c mice containing antidextran antibodies. In each case hemagglutination was totally inhibited. The same results were achieved in analogous experiments with 104E IgM-coated mouse red cells. Isolated normal mouse Igs or other myeloma proteins in amounts of 100–300 μg failed to inhibit this hemagglutination.

Immune response to dextran. The BALB/c mice immunized with α-1,3-dextran, had 123,026 (5.09 ± 0.04) antidextran antibody-producing cells per spleen. The number of splenic cells producing MOPC 104E IgM and J558 idiotypes were 165,958 (5.22 ± 0.02) and 70,794 (4.85 ± 0.10), respectively (Table I). These antidextran plaques were not completely inhibited by the antiidiotypic antibodies. The percentage inhibition for the MOPC 104E plaques was 38–45% and for J558 plaques 40–48%. The mice immunized with MOPC-104E IgM prior to dextran challenge, had quantitatively the same response to dextran as the controls (Table I). There were 128,824 (5.11 ± 0.04) antidextran-producing cells and their idiotype was predominantly that of J558: 81,283 (4.91 ± 0.03) plaques versus 105 (2.02 ± 0.47) 104E IgM idiotype-producing cells. Thus, there was idiotype suppression in excess of 99%. In mice immunized with J558 IgA, there was a slight but significant reduction in antidextran plaque-forming cells: 63,095 (4.8 ± 0.07) indicating a 41% suppression (Table I). There was a reduction of the cells making J558 idiotype to 1202 (3.08 ± 0.29) (99% suppression). The number of cells making 104E idiotype was 125,892 (5.10 ± 0.03). The BALB/c mice, which received one dose of complete Freund's adjuvant 10 days prior to dextran immunization, had a normal antidextran response, with both idiotypes represented (Table I). In MOPC 104E IgM-imunized mice, the percentage inhibition of antidextran plaque-forming cells with anti J558 was 73%, implying there still were 27% of cells which were producing dextran-specific idiotypes other than J558 IgA. In the case of the mice immunized with J558 IgA 70% of antidextran plaque-forming cells were inhibited by antiserum to MOPC 104E IgM. Again 30% of the cells with antidextran specificity must have produced other idiotypes.

The sum of the number of cells secreting 104E and J558 idiotypes was larger than the number of cells secreting the dextran antibodies. In order to explain this discrepancy

an attempt was made to inhibit the formation of plaques with either idiotype by dextran. In normal as well as in immunized mice, this inhibition was 100%. These results indicate that these plaques produced only dextran-specific idiotypes. It is likely that technical reasons, perhaps the indirect method of developing the plaque-forming cell in each instance are responsible for this discrepancy. Nevertheless, these experiments clearly showed the specific idiotype suppression.

Sheep RBC and LPS response. The SRBC response in mice immunized with 104E IgM or J558 IgA were 56 and 65% of the control response, and were significantly diminished ($P < 0.04$). However the effect of Freund's adjuvant was partially responsible for this diminished response (Table II). The LPS response in mice immunized with myeloma proteins was 113 and 95% of the control response, respectively (Table II).

Discussion. The results are in agreement with those of Carson and Weigert and indicate that B1335 dextran with α-1,3-glucosidic linkage elicits in the BALB/c mouse antidextran antibodies which contain at least two idiotypic specificities, corresponding to the myeloma protein J558 IgA-λ and MOPC 104E IgM-λ. When BALB/c were immunized with either one of these two myeloma proteins, the dextran response was preserved, but the idiotype of these antibodies was MOPC 104E IgM-λ in J558 IgA-λ-immunized mice, and J558 IgA-λ in MOPC 104E IgM-λ-immunized mice.

In the study of the effect of antiidiotypic antibodies on the control of B-cell responsiveness to antigens, other idiotypic systems have been used extensively. Nisonoff (8, 10, 11) and his associates have studied the immune response of AJ mice to *p*-azophenyl arsenate. Sixty to seventy percent of the mice respond to this antigen by producing cross-reactive idiotypes. Administration of antiidiotypic antibodies to this cross-reactive idiotype into AJ mice 2 weeks prior to the antigen challenge resulted in the suppression of the cross-reactive idiotype. However, these mice responded to this haptenic antigen by producing a new idiotype. The suppression was transferable to other AJ mice by ascitic fluid lymphocytes from idiotype-suppressed mice. Eichmann achieved the suppression of the anti-streptococcal A idiotype in AJ mice by prior injection of antiidiotypic antibodies. This idiotype suppression was transferable by T cells from suppressed mice (12, 13).

Kohler *et al.* (9, 14, 15) have shown that the antibody response of BALB/c mice to phosphorylcholine which is predominantly of the TEPC 15 IgA idiotype is suppressed by prior immunization of the mice with this myeloma globulin. However, recently Accolla *et al.* have shown that the neonatal suppression of the TEPC 15 IgA idiotype in BALB/c mice resulted in the appearance of a second idiotype similar to MOPC 167 IgG in response to immunization with phosphorylcholine (29). The absence of cross-reaction between the two idiotypes of antidextran antibodies speaks for the fact that these antibodies react with different epitopes of the antigen. Indeed, immunochemical studies by Leon *et al.* and Lundbald *et al.* provide evidence that MOPC 104E IgM was complementary to a hapten of the size of trisaccharides and J558 to a pentasaccharide (2, 30).

Whether the selective effect of antiidiotypic antibodies on the expression of idiotypes in antidextran antibodies is due to (a) antiidiotypic antibodies acting as antireceptor antibodies, (b) loss of specific B-lymphocyte clones, or (c) induction of specific suppressor T or B lymphocytes needs to be explored. Since there have been five clonotypes identified (7) for the antidextran response in BALB/c mice, our studies should also be expanded to the other three.

Summary. The two mouse myeloma globulins, MOPC-104E IgM and J558 IgA, have antibody specificity to α-1,3-dextran and represent two idiotypes of antidextran antibodies. The results of the reported experiments indicate that idiotype suppression could be achieved by prior immunization of BALB/c mice with either 104E IgM or J558 IgA. After this immunization antibodies to dextran were only of the other idiotype. The absence of cross-reactivity strongly suggests that the idiotypes of antidextran antibodies are directed against different epitopic groups on the antigen.

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