MECHANISM OF UNRESPONSIVENESS TO THE ALPHA 1–6 EPITOPE OF DEXTRAN B512 IN A C57BL SUBSTRAIN

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The ability to produce antibodies against the alpha 1–6 epitope of dextran B512 is determined by several genes. One (or several linked) gene(s) is located in the chromosome coding for the heavy chain of immunoglobulins and determines the ability of mice to produce antibodies against the alpha 1–6 epitope of dextran (4). This Igh-V gene is present in CBA, C57BL/10, and C57BL/6 mice, but is lacking in several other strains not having the Igh-C allotypes j and b. In another strain (CBA/N) an X-linked gene codes for the inability of this strain to mount immune responses against thymus-independent (TI)[1] antigens of type II, such as ficoll and dextran (5, 14, 15). The CBA/N mice possess the Igh-C–linked V gene coding for antibodies against the alpha 1–6 epitope of dextran (5), but appear to lack the B cell subpopulation that can be activated by TI II antigens or they lack a gene determining a B cell-activating receptor for TI II antigens (14, 15).

A substrain of C57BL, namely C57BL/10ScCr, has been found to be a low responder strain to lipopolysaccharide (LPS) and purified protein derivative of tuberculin (PPD) (2). Since C57BL/10 mice possess the Igh-C–linked V gene coding for antibodies against the alpha 1–6 epitope of dextran, it was expected that C57BL/10ScCr mice should also be able to produce antibodies against dextran. However, neither thymus-dependent nor thymus-independent forms of dextran could induce good antidextran responses in this substrain. We have now analyzed the mechanism responsible for this low response to dextran.

Materials and Methods

Mice. The inbred mouse strains C57BL/10, CBA, and A have been bred in our mouse colony since 1967. The C57BL/10ScCr strain was obtained from Bomholtgaard, Ry, Denmark in 1980 and has since been maintained in our department by brother sister matings.

Preparation of Cell Suspensions. Spleens were removed and teased with forceps in ice-cold balanced salt solution (BSS). After brief sedimentation, the cells in the supernatant were washed three times in 50 ml of cold BSS and then suspended to the desired cell concentration. Cellular viability was determined in a hemocytometer after the damaged

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[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; BSS, balanced salt solution; DNP, dinitrophenyl; HRBC, horse erythrocyte; i.v., intravenously; LPS, lipopolysaccharide; PBA, polyclonal B cell activator; PFC, plaque-forming cells; PPD, purified protein derivative of tuberculin; SRBC, sheep erythrocyte; TI, thymus-independent; TNP, trinitrobenzenesulphonic.

cells had been stained with 0.02% trypan blue.

Antigens and Immunizations. Native dextran from Leuconostoc mesenteroides B512, average molecular weight 40–100 × 10^6 daltons, was purchased from ICN Pharmaceuticals, Cleveland, OH. The mice were immunized with 10 μg of dextran given intravenously.

In addition, nonimmunogenic dextran mol wt 40,000 was conjugated to bovine serum albumin (BSA-dextran). This antigen was prepared as described before and its thymus dependence has been documented elsewhere (13). Mice were immunized intravenously (i.v.) with 80 μg of this antigen. Native dinitrophenyl (DNP) dextran was synthesized from native dextran by reacting it with *N*-epsilon-DNP-L-lysine HCL, using the method of Axén et al. (1). Finally horse erythrocytes cells (HRBC) or sheep erythrocytes (SRBC) were used as a 10% solution. The mice were given red cells i.v. in a volume of 0.1 ml.

Assay of Antibody Synthesis. Anti-alpha 1–6 plaque-forming cells (PFC) were detected by a direct assay using SRBC sensitized with stearoyl dextran B512 with a molecular weight of 70,000, as described before by Howard and co-workers (3). To detect anti-DNP PFC, trinitrobenzenesulphonic acid (TNP) was conjugated to SRBC.

In addition, indirect (IgG) PFC were developed as described elsewhere (8) using a rabbit anti-mouse immunoglobulin antiserum. In some experiments the protein A plaque assay was used, as described by Gronowicz et al. (12).

Detection of Antiidiotypic Antibodies. Sera from dextran immunized (10 μg of native dextran B512) mice were pooled 8 or more days after immunization. The sera were used after absorption with an equal volume of packed Sephadex G-75 in the cold over night to remove antidextran antibodies. The efficiency of absorption was verified by the disappearance of antidextran hemolytic antibodies as well as in a radioimmunoassay. The absorbed serum was assayed for its ability to suppress plaque-forming cells against dextran or HRBC by adding them in a final dilution of 1/1,000 to a plaque assay using dextran coated SRBC and HRBC as targets. The spleen cells used in the plaque assay were derived from mice that were immunized against dextran and HRBC 5 d earlier. The assays were done in duplicates.

Mitogens. Lipopolysaccharide from *Escherichia coli* O55:B5 was prepared by phenol-water extraction and was obtained from Dr. T. Holme, Department of Bacteriology, Karolinska Institute, Stockholm.

Dextran-sulphate was purchased from Pharmacia, Uppsala, Sweden and had a mol wt of 5 × 10^5. The conjugate had a sulphur content of 17% corresponding to an average of 2.3 sulphate groups per glucosyl residue and a nitrogen content lower than 0.001%.

Concanavalin A was obtained from Pharmacia, Uppsala, Sweden.

Culture Conditions. Spleen cells were cultured in two different systems, both using RPMI medium in the absence of serum. Induction of polyclonal antibody synthesis was performed in 3-cm-diameter plastic Petri dishes (Nunc, Denmark) with a cell concentration of 10^7 cells/ml per culture in 1-ml cultures, set up in triplicates. Cultures were incubated at 37°C in plastic boxes filled with a mixture of 10% CO_2, 83% N_2, and 7% O_2.

Induction of DNA synthesis was performed in Micro Test II tissue culture plates (no. 3040; Falcon Plastics, Oxnard, CA) with a cell concentration of 10^6 cells/ml in 0.2 ml cultures. These cultures were incubated in the same conditions as above. All mitogens were dissolved in the same medium as that used for culturing the cells.

Assay of DNA Synthesis. Cultures were given a 24-h pulse of [^{3}H]thymidine (The Radiochemical Centre, Amersham, England) with a specific activity of 5 Ci/mM, diluted in culture medium for a concentration of 2 μCi/ml in the cultures. Microcultures were harvested by means of a multiple cell culture harvester (Flow Laboratories, Scotland), washing each culture ~10 ml of cold distilled water.

Results

As can be seen in Table I both CBA and C57BL/10 mice gave a good response to the thymus-independent antigen native dextran B512, whereas C57BL/10ScCr gave a very poor response. One necessary prerequisite for B cells to be activated by a thymus-independent antigen is that they can respond to the

TABLE I

*Low Antibody Response Against Native Dextran in C57BL/10ScCr Mice**

Strain	No. of mice	IgM PFC/spleen
C57BL/10ScCr	37	16.453 ± 2.586
C57BL/10	15	124.053 ± 21.262
CBA	13	72.492 ± 13.106

* Mice older than 3 mo were immunized with 10 μg native dextran i.v. The PFC response was determined 5 d later.

TABLE II

*Both Thymus-independent and Thymus-dependent Forms of Dextran Fail to Induce a Strong Immune Response in C57BL/10ScCr**

Strain	PFC against dextran after immunization with: BSA-dextran		Native dextran	
	IgM	IgG + IgM	IgM	IgG + IgM
C57BL/10ScCr	960 ± 197	27 ± 13	3.387 ± 795	173 ± 74
CBA	9.533 ± 3.687	3.042 ± 2.042	23.040 ± 6.400	15.946 ± 6.676
C57BL/10	26.667 ± 8.030	68.480 ± 18.560	48.213 ± 14.675	131.328 ± 27.500

* The mice were immunized i.v. with 10 μg native dextran, 100 μg edestin-dextran, or 80 μg BSA-dextran and the immune response determined 5 d later.

TABLE III

*Induction of Polyclonal Antibody Synthesis in Lymphocytes from C57BL/10 and C57BL/10ScCr Mice by Different PBA's**

Polyclonal B cell activator	Dose	IgM PFC with cells from: C57BL/10		C57BL/10ScCr	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
	μg/ml				
—		20.267	79.350	3.067	4.480
LPS	100	130.130	158.720	5.000	19.840
Dextran	10.000	142.933	189.440	54.400	116.480
Dextran-sulphate	100	99.200	116.480	34.133	53.760

* Serum-free cultures were used containing 10^7 cells/ml in 1 ml cultures. The IgM PFC response was determined at day 2 using protein A-coated SRBC as targets and a rabbit anti-mouse IgM developing serum.

activating signal intrinsic to most thymus-independent antigens (6). It seemed possible that B cells from C57BL/10ScCr mice could lack activating receptors for dextran. If so they would respond to thymus-dependent forms of dextran, which activate another B cell subpopulation than thymus-independent antigens (13). For this purpose, nonimmunogenic forms of dextran (40,000 mol wt) was conjugated to BSA and used as an immunogen. As can be seen in Table II, the thymus-independent antigen induced an even smaller IgM and IgG immune response than did native dextran. CBA and C57BL/10 mice both gave good IgM responses, whereas only C57BL/10 mice exhibited IgG antibodies.

Response of Lymphocytes from C57BL/10ScCr to Polyclonal B Cell Activators

TABLE IV

*Induction of DNA Synthesis in Lymphocytes from C57BL/10 and C57BL/10ScCr Mice by Different Mitogens**

Mitogen added	Dose	[^{3}H]Thymidine incorporation (cpm) with cells from: C57BL/10		C57BL/10ScCr	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
	μg/ml				
—	—	14.049 ± 7.885	12.677 ± 886	2.113 ± 218	3.893 ± 439
LPS	100	62.540 ± 2.691	56.433 ± 1.976	7.023 ± 1.419	16.588 ± 2.190
Dextran	10.000	31.267 ± 1.409	41.130 ± 1.986	21.445 ± 414	20.725 ± 1.005
Dextran sulphate	100	83.113 ± 5.835	44.152 ± 504	55.469 ± 4.993	14.555 ± 433
Con A	0.5	55.914 ± 4.994	94.384 ± 9.637	44.780 ± 6.646	65.228 ± 8.191

* Serum free cultures containing 5 × 10^5 cells/well were used. [^{3}H]Thymidine was added between day 1 and 2.

TABLE V

*Anti-TNP Response in Normal and T × B Mice After Immunization with TNP-Dextran**

Strain	T × B‡	Anti-TNP PFC of the indicated affinities§		
		High	Medium	Low
C57BL/10	−	26.560 ± 6.000	58.720 ± 8.915	65.440 ± 10.360
C57BL/10ScCr	−	30.827 ± 9.539	58.240 ± 11.270	44.480 ± 12.470
C57BL/10ScCr	+	36.320 ± 10.090	65.120 ± 17.920	58.240 ± 15.819

* Three mice/group were immunized i.v. with 100 μg DNP-dextran and the direct PFC response tested 5 d later.

‡ T × B = thymectomized, lethally irradiated mice reconstituted with anti-theta-treated bone marrow cells.

§ The affinities were measured by the use of target red cells conjugated with 0.31 μg/ml for high, 1.25 μg/ml for medium 2.5 μg/ml of TNP for low affinity PFC.

TABLE VI

*Antidextran and Anti-HRBC Response in Lethally Irradiated and Bone Marrow Reconstituted Chimeras**

Chimera	Direct PFC response/spleen against:	
	Dextran	HRBC‡
C57BL/10ScCr into C57BL/10	7.296 ± 2.074	50.624
C57BL/10ScCr into C57BL/10ScCr	12.032 ± 2.030	70.784
C57BL/10 into C57BL/10	34.304 ± 10.497	61.312
C57BL/10 into C57BL/10ScCr	37.504 ± 8.155	55.040

* Five mice per group were immunized with 10 μg of native dextran mixed with HRBC and their direct PFC response determined 5 d later. The mice had 3 mo earlier been lethally irradiated and repopulated with 2 × 10^6 bone marrow cells of the indicated origin.

‡ The responses against HRBC were made with pooled suspension of spleen cells.

(PBA). One mechanism responsible for the failure of B cells to respond to certain antigens is that they lack receptors for different PBA (5, 17). In order to test whether this was the case with B cells from C57BL/10ScCr mice, they were activated in vitro with dextran and other PBA's. In addition, mice were injected

TABLE VII

C57BL/10ScCr Mice Possess the Igh-V Gene Coding for Antibodies Against the Alpha 1–6 Epitope of Dextran, since (A × C57BL/10ScCr)F_1 Hybrids are Responders to Dextran

Mice	No. of mice	IgM PFC against dextran
A	3	5.973 ± 5.335
(A × C57BL/10)F_1	3	55.133 ± 16.722
C57BL/10	3	42.133 ± 9.432
C57BL/10ScCr	3	7.200 ± 2.818
CBA	3	102.806 ± 18.778
(A × C57BL/10ScCr)F_1	3	50.773 ± 20.607

* The mice were immunized with 10 μg of native dextran i.v. and tested for direct antidextran PFC on day 5.

TABLE VIII

The Antidextran PFC Produced by (A × C57BL/10ScCr)F_1 Hybrids Share an Idiotype with PFC from High Responder C57BL/10 Mice

Auto-antiidiotypic serum donor strain	Serum dilution	Percent inhibition of PFC from the indicated strains after addition of auto-antiidiotypic serum*				
		(A × C57BL/10)F_1	C57BL/10	C57BL/10ScCr	CBA	(A × C57BL/10ScCr)F_1
CBA	1/1,000	0	10	0	39	10
C57BL/10	1/1,000	38	50	0	3	36

* Auto-antiidiotypic serum obtained 14 d after immunization with dextran was absorbed with Sephadex G-75 to remove antidextran antibodies and added to the plaque assay in a final dilution of 1/1,000. The cells used in the plaque assay were derived from mice immunized with dextran 5 d earlier.

with DNP-dextran and their anti-TNP response tested. In this case, the activation signal for TNP specific B cells is delivered by the dextran carrier. Therefore, the latter system also tests for the presence of B cell activation receptors for dextran.

Dextran was found to activate antibody synthesis (Table III) and DNA synthesis (Table IV) in lymphocytes from both C57BL/10 and C57BL/10ScCr mice. The response was lower than with C57BL/10 cells, but the background was also lower. LPS induced a low response in lymphocytes from C57BL/10ScCr mice, as reported before (2). Both strains responded to dextran sulphate, a PBA known to require macrophages and T cells for optimal B cell activation (10, 16).

The response to TNP after immunization with DNP-dextran was the same in C57BL/10 and C57BL/10ScCr mice (Table V). Therefore, C57BL/10ScCr mice do not lack the PBA receptor for dextran or the B cell population responsive to activation signals delivered by dextran.

Lymphocytes from C57BL/10ScCr Fail to Respond in Irradiated C57BL/10 Recipients. It seemed possible that lymphocytes from C57BL/10ScCr were prevented from responding to dextran, because they were actively suppressed by cells or environmental factors present in C57BL/10ScCr mice. Therefore, bone marrow cells were adoptively transferred from C57BL/10ScCr mice into lethally irradi-

ated C57BL/10, and, as a control into C57BL/10ScCr mice. Other controls included bone marrow cells from C57BL/10 mice transferred into lethally irradiated C57BL/10ScCr and C57BL/10 recipients, respectively. 3 mo after repopulation the mice were immunized with native dextran together with HRBC. As can be seen in Table VI C57BL/10ScCr cells transferred into C57BL/10 recipients still failed to respond to dextran, whereas C57BL/10 cells responded to dextran both in syngeneic and C57BL/10ScCr recipients. All recipients responded equally well to HRBC in any recipient.

Complementation of the Response to Dextran in F_1 Hybrids between C57BL/10 ScCr and A Mice. C57BL/10ScCr is expected to possess the Igh-V gene coding for antibodies against dextran, since other C57BL/10 and C57BL/6 substrains express this gene (4). However, we tested whether this was the case by immunizing (A × C57BL/10ScCr)F_1 hybrids with dextran. In this hybrid, strain A does not possess (or does not express) the antidextran Igh-V gene and cannot produce antibodies against dextran (5). If the hybrid responds, the Igh-V gene against dextran must come from the C57BL/10ScCr haplotype. As can be seen in Table VII, this F_1 hybrid responded well to dextran.

The Antidextran PFC Produced by (A × C57BL/10ScCr)F_1 Hybrids Share Idiotype with PFC from High Responder C57BL/10 Mice. The ability of (A × C57BL/10ScCr)F_1 hybrids to respond to dextran does not necessarily imply that the same Igh-V gene is expressed in these hybrids and high responder C57BL/10 mice. In order to test this, we suppressed the antidextran PFC by adding auto-antiidiotypic antibodies obtained from CBA and C57BL/10 mice, respectively to the PFC assay. We have shown before (9, 11) that auto-antiidiotypic antibodies appear spontaneously during the immune response to dextran. CBA mice produce auto-antiidiotypic antibodies that suppress PFC from CBA, but not from C57BL/10 mice, and the reverse.

Auto-antiidiotypic sera from CBA and C57BL/10 mice were collected 14 d after a primary antidextran response and absorbed with Sephadex G-75 to remove antidextran antibodies. The auto-antiidiotypic sera were then added to a plaque assay using spleen cells from mice immunized against dextran 5 d earlier. As can be seen in Table VIII, auto-antiidiotypic sera from CBA mice suppressed antidextran PFC from CBA, but not from C57BL/10 or (A × C57BL/10ScCr)F_1 mice, whereas auto-antiidiotypic sera from the latter showed the reverse pattern. Thus, antidextran PFC from C57BL/10 and C57BL/10ScCr mice share a common idiotype.

Discussion

The low responsiveness of C57BL/10ScCr mice to dextran was not due to lack of PBA receptors for dextran, since native dextran induced polyclonal B cell activation and DNP-dextran was a good immunogen for induction of anti-TNP antibodies. Furthermore, the Igh-C-linked dextran V gene was expressed in C57BL/10ScCr mice, since the F_1 hybrids (A × C57BL/10ScCr) responded well to dextran. In this hybrid the V gene coding for antibodies against dextran comes from the C57BL/10ScCr haplotype, since A mice lack (or do not express) the dextran Igh-V gene. In addition, the antidextran PFC produced in (A × C57BL/10ScCr)F_1 hybrids and C57BL/10 mice shared a common idiotype. In

addition, low responsiveness to dextran seems to be a property of the C57BL/10ScCr lymphocytes, since transfer into irradiated high responder recipients did not alter the response. Finally, tolerance can also be discarded as the mechanism of unresponsiveness of C57BL/10ScCr mice, since bone marrow lymphocytes from C57BL/10 mice were not prevented from responding to dextran when transferred into C57BL/10ScCr mice.

The low response to dextran that was observed in C57BL/10ScCr mice appears to be different from that of high responder C57BL/10 mice, since the antidextran PFC produced by lymphocytes from C57BL/10ScCr mice were not suppressed by auto-antiidiotypic antibodies from C57BL/10 mice.

Since dextran was a good carrier for the induction of anti-TNP responses in C57BL/10ScCr mice, the defective response was not due to a general inability to produce antibodies against TI II antigens, as is the case with B cells from the CBA/N strain. Therefore, it seems as the low responsiveness to dextran of C57BL/10ScCr mice is caused by a determinant specific defect. Such a situation has been observed before using dextran as an immunogen. Thus, the very late ontogenic development of immunocompetence against dextran (7) and the inability of CBA mice to produce IgG antibodies against dextran (8) both specifically affected the alpha 1–6 epitope of dextran and not other epitopes conjugated to dextran as a carrier.

The exact nature of this determinant specific immune defect against the alpha 1–6 epitope of dextran in C57BL/10ScCr mice remains unknown, but suggests the existence of as yet unknown regulatory influences of Igh-V gene expression on B cell activation.

Summary

C57BL/10ScCr mice are low responders to the alpha 1–6 epitope of dextran B512, although other C57BL mice are high responders. Both thymus-independent and thymus-dependent forms of dextran failed to induce an immune response in C57BL/10ScCr mice, but dextran functioned as a good carrier for antihapten responses in this strain. Dextran is a potent polyclonal B cell activator for cells from C57BL/10ScCr mice, although such cells are not activated by LPS. The C57BL/10ScCr mice possess the Igh-V gene coding for antibodies against dextran and the antidextran antibodies induced in (A × C57BL/10ScCr)F_1 hybrids share an idiotype with antidextran antibodies produced in C57BL/10 mice. Bone marrow cells from C57BL/10ScCr mice do not respond to dextran when transferred into lethally irradiated C57BL/10 mice and C57BL/10 cells transferred into C57BL/10ScCr mice give a strong antidextran response.

Thus, B cells having both the Igh-V gene coding for antibodies against dextran and activation receptors for dextran cannot be activated into antibody synthesis against any form of this immunogen. This determinant specific immunodeficiency suggests the existence of as yet unknown regulatory influences on Igh-V gene expression or B cell activation.

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