IMMUNOLOGICAL UNRESPONSIVENESS TO
THYMUS-INDEPENDENT ANTIGENS:
TWO FUNDAMENTALLY DIFFERENT GENETIC
MECHANISMS OF
B-CELL UNRESPONSIVENESS TO DEXTRAN*

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It is well established that the ability to respond to certain antigens is under genetic
control and several different types of control mechanisms are known. The genetic
control of responses to thymus-dependent (TD) antigens usually affects IgG synthesis
and two separate types have been observed. The immune response to certain antigens is
under control of antigen-specific immune response genes, which are localized in the
chromosome region determining the major histocompatibility antigens and they exert
their effect on thymus-derived lymphocytes (1). These genes do not code for the variable
region of the immunoglobulin receptors on the B cells and a normal response can be
induced provided adequate T-cell help is given. A second type of genetic control to TD
antigens is not antigen specific, only effects the quantity of the immune response, and is
not controlled by histocompatibility-linked genes (2).

The immune response to thymus-independent (TI) antigens can also be under genetic
control and also in this case two mechanisms, which primarily affect IgM synthesis, can
be distinguished. One of these mechanisms concerns the ability of B cells to become
activated by the polyclonal B-cell activating (PBA) properties inherent to TI antigens.
In one case (C3H/HeJ), the gene(s) determine(s) the presence of the nonimmunoglobulin
receptors on B cells for lipopolysaccharide, which are responsible for B-cell activation.
The genes are codominantly expressed (3, 4) and localized on chromosome 4 in the
mouse. They do not code for the variable part of the immunoglobulin receptors.
Another nonresponding strain to certain TI antigens (CBA/N) lacks PBA receptors or
receptor-bearing cells capable of reacting to, for example, Ficoll, and cannot mount an
immune response to any hapten coupled to this TI carrier (5). The gene is localized on
the X chromosome. A second type of genetic control of the response to TI antigens con-
cerns the α-1-3 epitope of dextran (Dx) (6). The gene(s) determine(s) low immune re-

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1 Abbreviations used in this paper: BSS, balanced salt solution; Con A, concanavalin A; Dx,
dextran; FITC, fluorescein isothiocyanate; HRC, horse erythrocytes; NNP, (4-hydroxy-3,5-dinitro-
phenyl)acetyl; PBA, polyclonal B-cell activator; PFC, plaque-forming cells; PHA, phytohemag-
glutinin; RBC, erythrocytes; SRBC, sheep erythrocytes; TD, thymus dependent; TI, thymus
independent.

2 J. Watson, K. Kelly, M. Largen, and B. A. Taylor. The genetic mapping of a defective LPS
response gene in C3H/HeJ mice. Manuscript submitted for publication.

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sponsiveness rather than absence of a response and at least one of the genes is closely linked to allotypes of the heavy chain locus (7).

We have found that different mouse strains vary in their ability to produce antibodies to the α-1-6 epitope of Dx B512, and in certain strains a large proportion of mice are total nonresponders (8). Since Dx activates B cells in the absence of helper cells, such as T cells and macrophages (9), and since suppressor T cells do not affect this response (10), only two fundamental mechanisms can account for unresponsiveness, specifically: (a) lack of expression of V genes coding for antibodies against the α-1-6 epitope and (b) lack of triggering receptors on the B cells possessing immunoglobulin receptors for the epitope. We will show that both types of unresponsiveness can occur in different mouse strains.

Materials and Methods

Mice of the following strains were used in the present study: A/Sn, A.CA, A.SW, BALB/c, CBA, CBA/N, C3H/Tif, C3H/Hsd, C57BL, A.TH, A.TL, 4R, 2R, B10.5M, and certain hybrids among these strains.

Antigens and Polyclonal Activators. Native Dx from Lactobacillus mesenteroides B512 (average mol wt 5–40 × 10^3 daltons), was obtained from ICN Pharmaceuticals Inc., Cleveland, Ohio. Other Dx preparations were obtained from Pharmacia, Uppsala, Sweden.

Native fluorescein isothiocyanate (FITC)-Dx was synthetized from native Dx B512 by reacting it with FITC, and was provided by Dr. van de Belder, Pharmacia. The final conjugation ratio was one molecule of FITC for every 200 glucose residues.

Lipopolysaccharide from Escherichia coli 055:B5 was prepared from phenol water extraction provided by professor T. Holme (Department of Bacteriology, Karolinska Institutet).

Concanavalin A (ConA) and phytohemagglutinin (PHA) were obtained from Pharmacia, Uppsala, Sweden, and Wellcome Reagents Limited, Beckenham, England, respectively.

Preparation of Lymphocytes. Spleens were removed and teased with forceps in ice-cold balanced salt solution (BSS). After brief sedimentation, the cells in the supernate were washed three times in 50 ml of cold BSS and subsequently suspended in culture medium to the desired cell concentration. Cellular viability was determined in a hemocytometer after staining the damaged cells with 0.02% trypan blue.

Assay of Antibody Synthesis. Anti-α-1-6 plaque-forming cells (PFC) were detected by a direct PFC assay with sheep erythrocytes (SRBC) with stearyl Dx B512 with a mol wt of 70,000 daltons, as described before by Howard et al. (11).

Anti-FITC-PFC. The coupling of FITC to erythrocytes (RBC) has been described before (12). For detection of high, medium, and low affinity PFC the following concentrations of FITC in carbonate bicarbonate buffer pH 9.2 were used: 0.05, (of 0.1) 0.5, and 5 mg/ml.

Anti-NNP PFC. RBC were coupled with (4-hydroxy-3,5-dinitrophenyl)acetyl (NNP) as described by Pasanen and Mäkelä (13). These cells were used to detect polyclonal antibody synthesis.

Medium. The medium used in most of the experiments was Eagle's Minimum Essential Medium in Earle's solution, supplemented with glutamine, non-essential amino acids, and pyruvate, and containing 100 IU of penicillin and 100 µg of streptomycin/ml, as described by Mishell and Dutton (15). The medium was further buffered by 10 mM of HEPES and the pH adjusted to 7.2. All these reagents were obtained from Flow Laboratories, Irvine, Scotland. Most experiments were carried out in serum-free medium (14), except where specifically indicated in the figure legends.

Induction of Polyclonal Antibody Synthesis. Spleen cells were cultured serum-free in 3-cm diameter plastic Petri dishes or tubes (NUNC, Roskilde, Denmark) with a cell concentration of 10^6 cells/ml in 1 ml medium (15) set up in triplicate unless otherwise stated. The cultures were incubated at 37°C in plastic boxes filled with a mixture of 10% CO₂, 8% N₂, and 7% O₂.
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Table I

| Strain | No. of mice | PFC/spleen against SRBC | α-1-6* |
|--------|-------------|-------------------------|--------|
| CBA    | 16          | 90 ± 18                 | 33,340 ± 4,900 |
| C57BL  | 8           | 330 ± 100               | 46,440 ± 6,900 |
| A.CA   | 8           | 178 ± 52                | 2,778 ± 1,679 |
| A      | 17          | 105 ± 34                | 2,786 ± 695  |
| A.TH   | 11          | 11 ± 9                  | 1,036 ± 770  |
| A.TL   | 9           | 33 ± 15                 | 788 ± 377   |
| CBA/N  | 6           | 2 ± 1                   | 5 ± 3      |

* The PFC response against α-1-6 was tested against SRBC coated with stearoyl Dy (40 µg in 10 ml) to detect PFC of medium affinity.

Results

Strain Variability in the Response to the α-1-6 Epitope. The immune response to native Dy B512 (α-1-6) was tested in 14 inbred strains and was found to be very strain dependent. High responder strains, such as C57BL and CBA gave 400–1,000 PFC/10^6 cells 5 days after immunization with the optimal dose 1–2 µg/mouse. In contrast, A, A.CA, A.SW, A.TH, A.TL, and CBA/N mice were found to be low or nonresponders to the α-1-6 epitope (8). Over 200 A.CA were tested and about 90% of them only gave 0–10 PFC/10^6 cells, which, at best, can be classified as an extremely low response. Other mouse strains, such as BALB/c were intermediate responders, exhibiting PFC values between the extremes reported above. Occasionally, A.CA mice exhibited a high response in terms of the number of PFC. This response was of very low affinity and the causes for this will be the topic of a separate report. A variable of importance in determining the level of the response was the age of the mice; young mice of all strains were nonresponders. However, when comparisons were made with mice of the same age (3–4 mo) the strain differences still existed (Table I). Since the mechanism of low responsiveness was found to be different with strains A, A.CA, A.SW, A.TH, and A.TL as compared with strain CBA/N, they will be dealt with separately.

Mechanism of Unresponsiveness in Strains on A Background. The low responsiveness of strains on A background could not be attributed to differences in dose requirements, affinity, or kinetics of the immune response, since A.CA mice failed to respond to the α-1-6 epitope over a wide range of Dy or FITC Dy concentrations and at any time after immunization (Table II). Nonresponsiveness also affected the immune response to Dy conjugated to the TD carrier horse erythrocytes (HRC) (Table III) even in the presence of PHA as a polyclonal T-cell activator. A.CA mice did not produce anti-α-1-6 antibodies of noncomplement fixing classes, since sera from A.CA mice did not agglutinate Dy-coated RBC. The incorporation of native Dy with Freund's adjuvant did not make the mice responsive.

Low Responsiveness is Not Due to Suppressor Cells. The existence of
### Table II
Dose Dependence, Kinetics, and Affinity of the Immune Response to Native Dx or Dx T 2000* in High and Low Responder Strains

| Strain | Antigen | Dose (µg/mouse) | Day of test | PFC/10⁵ against α-1-6 by using the following indicator* cells |
|--------|---------|-----------------|-------------|-------------------------------------------------------------|
|        |         |                 |             | 4   | 40 | 400 |
| CBA    | Native Dx | 1 4  | 228  | 289 | 268 |
|        |          | 10  | 174  | 541 | 289 |
|        |          | 100 | 54   | 92  | 72  |
|        |          | 1  6 | 131  | 339 | -   |
|        |          | 10  | 100  | 108 | 133 |
|        |          | 100 | -    | 92  | 60  |
|        |          | 1  15| 64   | 183 | 143 |
|        |          | 10  | 29   | 208 | 248 |
|        |          | 100 | -    | -   | -   |
| A.CA   | Native Dx | 1 4  | 1    | 2   | 1   |
|        |          | 10  | 3    | 2   | 6   |
|        |          | 100 | 4    | 4   | 8   |
|        |          | 1  6 | 2    | 4   | 1   |
|        |          | 10  | 5    | 2   | 13  |
|        |          | 100 | 1    | 2   | 1   |
|        |          | 1  15| 1    | 1   | 3   |
|        |          | 10  | 10   | 10  |
|        |          | 100 | 1    | 1   | 6   |
| CBA    | T 2000   | 1 4  | 192  | 217 | 129 |
|        |          | 10  | 202  | 327 | 199 |
|        |          | 100 | 47   | 127 | 107 |
|        |          | 1  6 | 270  | 237 | 210 |
|        |          | 10  | 151  | 239 | 150 |
|        |          | 100 | 230  | -   | 180 |
|        |          | 1  15| 113  | 223 | 165 |
|        |          | 10  | 105  | 327 | 646 |
|        |          | 100 | 17   | 446 | 757 |
| A.CA   | T 2000   | 1 4  | 5    | -   | 3   |
|        |          | 10  | 0    | 0   |
|        |          | 100 | 6    | 4   | 4   |
|        |          | 1  6 | 0    | 1   | 0   |
|        |          | 10  | 2    | 1   | 0   |
|        |          | 100 | 1    | 4   | 2   |
|        |          | 1  15| 2    | 28  | 41  |
|        |          | 10  | 1    | 0   | 1   |
|        |          | 100 | 1    | 28  | 31  |

*Mol wt = 2 × 10⁶.
† SRBC coated with 4, 40, or 400 µg/10 ml of stearoyl Dx during sensitization.

Suppressor T cells capable of specifically suppressing an immune response has been reported in several different systems (16). Suppressor T cells were not involved here, since thymectomized, lethally irradiated A.CA mice that had been repopulated with syngeneic anti-theta serum-treated bone marrow failed to respond to native Dx (reference 8 and not shown).

It still seemed possible that the environment of A.CA mice contained suppressor elements for the anti-α-1-6 responding cells. To test this possibility,
A.CA mice were lethally irradiated and repopulated with spleen cells from a high responder strain (B10.5M or CBA) and immunized at the time of repopulation with native Dx and as a control with HRC. In addition, spleen cells from A.CA mice were transferred into lethally irradiated high responder mice (B10.5M or CBA). The results (Table IV) showed that spleen cells from high responder strains responded well to native Dx in the A.CA environment, whereas spleen cells from A.CA mice failed to respond even in the high responder strains. Both groups responded to HRC. Thus, it appears unlikely that any type of specific suppressor influence can account for the lack of immune reactivity against the \( \alpha-1-6 \) epitope in A.CA mice.

**Dx is an Efficient Carrier in Dx Low Responsive Mice.** Lack of an immune response to TI antigens could be theoretically caused by two fundamental mechanisms (since suppressive effects have been excluded) specifically: (a) lack of PBA receptors on the responding B cells, which therefore cannot be activated after binding the antigens or (b) absence of Ig receptors competent to passively focus the antigen to the responding B cells. In the latter case the cells cannot produce antibodies, even if given the correct triggering signal via the PBA receptors.

The possibility that A.CA mice lack the PBA receptor for Dx was tested in two ways. First, it was investigated if Dx could induce polyclonal activation in these mice, as determined by induction of antibodies to unrelated antigens. Second, it was studied if Dx could act as a carrier for an unrelated hapten in a dextran nonresponder strain. Since activation of B cells is caused by signals
Immune Response to α-1-6 and HRC in Irradiated Recipients Reconstituted with Spleen Cells from High and Low Responder Strains

| Donor | Irradiated recipients | PFC/10^6 spleen cells against |
|-------|----------------------|-------------------------------|
|       |                      | α-1-6 | HRC | NNP |
| CBA   | CBA                  | 165   | 320 | 95  |
| ACA   | CBA                  | 14    | 84  | 76  |
| ACA   | ACA                  | 0     | 62  | 218 |
| CBA   | ACA                  | 139   | 268 | 116 |
| 5M    | ACA                  | 1,544 | 2,500 | 734 |
| ACA   | 5M                   | 6     | 340 | 179 |
| ACA   | ACA                  | 2     | 183 | 127 |

* The irradiated recipients (750 R) were given 30 × 10^6 spleen cell i.v. and were immediately immunized with 1 μg native Dx together with 0.2 ml of a 10% suspension of HRC. The PFC against the immunizing antigens as well as against SRBC and the hapten NNP were determined 4 days (CBA) or 5 days (5M) later.

Given to the cells by polyclonal B-cell activating properties of the TI antigen, Dx should not be able to work as a carrier for unrelated hapten; if the mechanism of unresponsiveness was lack of PBA receptors for Dx. The results were clear in both systems and showed that Dx was as potent PBA also in ACA mice. Thus, Dx induced polyclonal antibody synthesis in ACA mice in vivo or in vitro as it did in high responder strains (Figs. 1 and 2).

When the hapten FITC was conjugated to Dx and the conjugate used to immunize ACA mice as well as high responder strains, it was found that the conjugation of FITC to Dx did not make the ACA mice respond to α-1-6 (Fig. 3), in agreement with the findings that conjugation of Dx to HRC did not make ACA mice competent to respond to the α-1-6 epitope. However, ACA mice responded strongly to the FITC epitope and were equal in this respect to the α-1-6 high responder strains (Fig. 3). Thus, Dx can work as an efficient carrier for unrelated hapten, indicating that ACA mice possess PBA receptors for Dx.

**Polyclonal Activation of Cells from Low Responder Mice Does Not Induce Anti-α-1-6 Antibodies.** The second alternative given above (lack of V gene expression against the α-1-6 epitope) can be tested by the use of polyclonal B-cell activators, since these substances are competent to reveal the V gene repertoire of the B cells that are susceptible to activation by a particular PBA. This approach has been used successfully to study the presence of immunocompetent cells against the tolerogen in specifically tolerant animals (17) and even to induce autoantibody formation in resting B lymphocytes (18).

Consequently, polyclonal concentrations of the PBA lipopolysaccharide (LPS) were injected into ACA mice, (or added to such cells in culture) and as a control also injected into strains that were high responders to the α-1-6 epitope (or added to such cells in vitro). The groups were tested at various time intervals against different antigens (SRBC, HRC, NNP, FITC, and α-1-6 of high and medium affinity). The results showed that LPS was competent to induce antibody synthesis against all epitopes studied in the high responder strains, but ACA and A.TH mice consistently failed to express anti-α-1-6 PFC,
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FIG. 1. Induction of polyclonal antibody synthesis by 2 mg native Dx in spleen cells from A.CA and A × C57BL mice. 10⁷ spleen cells were cultivated in serum-free medium in the presence or absence of native dx for 2 days and thereafter assayed against FITC-SRBC by using a high epitope density. Bg indicates background in nonimmunized animals.

![Graph showing antibody synthesis](image)

FIG. 2. Induction of polyclonal antibody synthesis by different concentrations of native Dx in vitro by using spleen cells from A.CA (■) and C57BL (□) mice. The response was tested after 2 days in culture by using the culture system described in Fig. 1 against NNP-coated target cells.

![Graph showing antibody synthesis](image)

even though a PFC response could easily be detected against all other antigens used (Fig. 4 and Table V).

Analogous experiments carried out in vivo with strains A and CBA mice gave similar results (Fig. 5).

CBA/N Mice Lack the Major Part of the B-cell Population Responding to the PBA Property of Dx. A similar analysis was carried out with CBA/N mice to determine the mechanism of unresponsiveness to α-1-6. The results were
FIG. 3. Immune response against α-1-6 and FITC in A × CBA F₁ and A.CA mice after immunization i.v. with 100 μg/mouse of native FITC-Dx B512. The response was determined at day 5 by using the Jerne plaque assay as modified for the detection of PF Cells against α-1-6. For detection of anti-α-1-6 PFC, SRBC were conjugated with 40 μg of stearoyl Dx in 10 ml of a 5% suspension of SRBC. Anti-FITC PFC were detected with SRBC conjugated with a solution of 5 mg/ml of FITC.

FIG. 4. Induction of polyclonal antibody synthesis in vivo by injection of 100 μg of LPS i.p. into B10.5M and A.CA mice. The spleens were taken out after 2 and 4 days and tested for the number of PFC against α-1-6 and the hapten NNP. The response against α-1-6 was undetectable in A.CA mice.

completely different from those obtained with strains on A background. Thus, native Dx induced a very small polyclonal antibody response against the haptens FITC, NNP, or heterologous RBC (Fig. 6). Also the response to LPS was impaired, but was stronger than the response to Dx.

When CBA/N mice (or CBA × DBA F₁ males) were immunized with FITC-Dx they neither produced PFC nor α-1-6 against FITC. However, they gave a
TABLE V

Polyclonally Activating Concentrations of LPS Fail to Induce Anti-α-1-6 PFC in Cells from Low Responder Strains In Vitro*

| Strain | Treatment of cultures† | PFC/10² cells against the indicated targets at day |
|--------|-------------------------|--------------------------------------------------|
|        |                        | 3       | 6       |
| C57BL  | α-1-6§ FITC            | 0 55    | 0 100   |
|        | LPS                    | 380 2,042 | 600 5,000 |
| A.CA   |                         | 0 10    | 0 67    |
|        | LPS                    | 0 1,410 | 16 1,766 |
| A.TH   |                         | 0 30    | 0 17    |
|        | LPS                    | 0 880   | 0 1,700 |

* The lymphocytes were cultivated in 2 ml medium containing 5% human A serum and 5 x 10⁻⁵M 2-mercaptoethanol at a cell concentration of 3 x 10⁵ cells/ml.
† 100 μg/ml of LPS.
§ Stearoyl Dx at a concentration of 40 μg/10 ml was used to sensitize SRC as targets in the PFC assay.
∥ 5 mg/ml of FITC was used to sensitize SRBC as targets in the PFC assay.

Fig. 5. Induction of polyclonal antibody synthesis in vivo by the injection of 100 μg LPS into A and CBA mice. Their PFC response against α-1-6 and NNP was followed up to day 6. A mice (■) responded only to NNP (—-—) and not to α-1-6 (——), whereas CBA mice (△) responded to both epitopes.

definite, although small response against FITC when the hapten was conjugated to TD HRC, indicating that the mice possess V genes for the FITC epitope (Table VI). Thus, the cause for the unresponsiveness to FITC-Dx in this case appears to be the lack of the major part of the B-cell population having PBA receptors for Dx, in analogy with the findings that these mice lack triggering receptors for other PBA, such as Ficoll. CBA/N mice are analogous in this way
FIG. 6. Polyclonal activation by LPS and native Dx of spleen cells from CBA/N × DBA male and female mice. Half of the mice were untreated, whereas the other half were immunized 5 days earlier with 100 μg of native FITC-Dx and were tested for PFC response against FITC. Only the immunized females developed an immune response to FITC, the males were totally unresponsive. The spleen cells were cultivated in RPMI medium containing 5% fetal calf serum and 5 x 10⁻⁵ M 2-mercaptoethanol by using 2 x 10⁶ cells/ml and 0.5 ml per culture tube. LPS (50 μg/ml) and native Dx (4 mg/ml) were added to the indicated cultures. The cells were harvested on day 3 and tested against NNP-SRBC. Bg indicates background in nonactivated cultures.

TABLE VI

| Strain     | Antigen | SRBC | HRC  | PFC/spleen against α-1-6 | FITC1 |
|------------|---------|------|------|--------------------------|-------|
|            |         |      |      | 40                        | 400   | 0.2 | 5   |
| CBA/N      | -       | 10   | 20   | 0                         | 0     | 120 | -   |
|            | FITC-Dx | 5 ± 5| NT   | 10 ± 10                   | 0     | 45  | 45  |
|            | FITC-HRC| 35 ± 17| 3,625 ± 880 | NT    | NT | 1,490 ± 630 | 2,615 ± 660 |
| BALB/c    | -       | 25   | 50   | 25                        | 435   | 175 | 1,250 |
|            | FITC-Dx | 116 ± 116 | 1,033 ± 1,137 | 1,816 ± 1,236 | 30,223 ± 12,700 | 46,006 ± 20,500 |
|            | FITC-HRC| 200 ± 57| 93,000 ± 30,324 | NT | NT | 35,666 ± 9,700 | 50,530 ± 13,200 |

* The mice were immunized with 100 μg native FITC-Dx i.v. and with 0.2 ml of a 10% suspension of FITC-HRC labeled with 0.5 mg/ml of FITC.

† 40 and 400 μg/10 ml stearoyl Dx were used to coat SRBC.

‡ 0.2 and 5 mg/ml FITC were used to coat SRBC.

¶ Four mice/group.

* Three mice/group.

to the LPS nonresponder C3H/HeJ, lacking PBA receptors for LPS, but the defect is apparently more pronounced and affects cells with receptors for several different PBA and the genetic localization of the defect is different (it is located on the X chromosome).

Finally it was tested if CBA/N mice could produce anti-α-1-6 PFC after immunization with the TD conjugate Edistine-Dx (a plant protein conjugated
to Dx and provided by Dr. Richter, Pharmacia, Uppsala, Sweden). However, no detectable response was obtained.

Discussion

The findings in this paper illustrate two fundamentally different causes of immunological unresponsiveness at the B-cell level to the same epitope on Dx. Strains on A background were nonresponders because they failed to express V genes coding for antibodies against α-1-6. In contrast, strain CBA/N failed to respond to Dx or FITC-Dx, because the cell population having PBA receptors for Dx was severely depleted. This strain is therefore analogous to C3H/HeJ which cannot respond to LPS or any hapten coupled to LPS, because there is a selective loss of PBA receptors for LPS. CBA/N not only has defective B cells capable of responding to the PBA property of Dx, as shown here, but also cells responding to Ficoll, pneumococcal polysaccharide SIII, and polyacrylamide.

It has been claimed that this strain responds normally to haptenated LPS, indicating that it possesses B cells with PBA receptors for LPS (5). CBA/N responded, although poorly, to the PBA property of LPS. Although C3H/HeJ and CBA/N principally exhibit analogous defects, there are marked differences between the two strains. The defect in C3H/HeJ is selective for LPS, and is determined by one gene localized on the fourth chromosome, whereas CBA/N lacks receptors or B-cell populations for several different PBA's and the defect is X-linked. It could not be formally established whether CBA/N mice possess V genes for α-1-6, since they did not respond to TD Dx conjugates.

The fact that A mice did not respond to Dx, although they have cells with PBA receptors for Dx, is a new type of immunological unresponsiveness. It is a genetically determined (unpublished observations) inability to respond, which could not be ascribed to lack of helper T cells, presence of suppressor T cells, or any suppressive influence of the environment of the mice that did not allow the expression of an anti-α-1-6 response. The unresponsive state exhibited stringent immunological specificity, since only the α-1-6 epitope was affected, whereas other epitopes conjugated to Dx induced normal immune responses. Since it was not possible to convert nonresponders into responders by a variety of procedures that engage different helper cells, such as conjugating Dx to TD HRC, injecting it with polyclonal T-cell activators such as PHA and Con A, or incorporating it into Freund's adjuvant, it is unlikely that the defect can be ascribed to lack of helper cells. Furthermore, several different PBA's such as LPS and PPD, which could activate polyclonal antibody synthesis to all other antigenic determinants failed to do so against α-1-6 in the nonresponder strains, whereas expected anti-α-1-6 responses occurred with similarly-treated cells from high responder strains. It follows from these findings that B cells which can be activated by helper T cells, LPS, PPD, and Dx cannot express Ig molecules with the α-1-6 variable region. Since these polyclonal activators probably affect a major part of the B-cell population (LPS alone activates about 30% of all B cells and these are different from those activated by PPD and Dx) it follows that most if not all B cells do not express the gene responsible for the formation of the combining site for α-1-6.

The lack of detectable anti-α-1-6 antibody-producing cells could be due to two
main reasons. It is possible that an α-1-6 clone or several such clones are present in the nonresponders, but the number of cells in the clones is too small to be detected. Alternatively, lymphocytes from these animals either lack V genes coding for antibodies against α-1-6 or the cells cannot selectively express these V genes. Although the possibility that the clone size is too small to allow detection may be used to explain the in vitro data with PBA, it is highly unlikely that it is applicable for the in vivo experiments, since the amplification of the response was very pronounced in responder strains, which presumably have very few clones because of the homogeneity of the affinity of the antibodies produced. Usually the number of PFC against α-1-6 was 500-2,000 times the background 5 days after immunization. The exponential increase of the immune response during the first 5 days is predominantly due to division of initially activated precursor cells rather than to recruitment of new precursor cells. Actually it is possible to detect one precursor cell in adoptive transfer experiments and there is no reason why this would not also be possible against α-1-6 in vivo with the marked clonal expansion that occurs in high responders. There is no reason why precursor cells could not multiply in nonresponder strains; since no suppressor cells have been detected, there is not a need for helper cells, and other epitopes coupled to Dx induce a normal anti-hapten response.

The only tenable conclusion is that strains on A background fail to express Ig receptors and secrete antibodies directed against the α-1-6 epitope in the majority of the individuals. The mechanism by which a few individuals secrete antibodies against this epitope will be discussed below. It seems highly unlikely that some control mechanism would exist that selectively prevents the synthesis from messenger RNA of antibodies directed only against one particular epitope. It is more plausible that the defect is at the DNA level. Two mechanisms could account for the genetically determined state of unresponsiveness to Dx at the DNA level, namely lack of V genes coding for antibodies against α-1-6 or lack of V-C gene translocation for this particular specificity. Neither alternative has precedence, but both are possible. The possibility that different V genes would translocate at different rates (or not at all) is compatible with our findings (unpublished observations) that high responder strains to the α-1-6 epitope do not express this antibody during the 1st mo of life, even when the cells are polyclonally activated, for example, by LPS, whereas they express antibodies of all other specificities. This suggests that the V-C translocation can occur at different times for different V genes. It is possible that the V gene coding for anti-α-1-6 actually exists in strains on A background, but that it never, or rarely, translocates to a C gene. Conclusive experiments on this point require experiments at the DNA level.

A certain proportion of mice on strain A background produced antibodies against α-1-6, in most cases to a very small degree. Usually the PFC differed markedly from those in high responder strains, because they were unclear and of very low affinity, since they were detected easily and in high numbers only with target cells coated with a high epitope density. This is in contrast to the PFC obtained in high responder strains, which were of uniform affinity; no difference in number or clarity was observed with target cells of different epitope density, suggesting a mono- or pauciclonal response. Mice lacking a
particular V gene may occasionally make use of another V gene, which may have mutated so as to cause the appearance of an antibody crossreacting with the epitope for which a V gene is lacking. This may very well be the case with the small proportion of animals responding to the α-1-6 epitope in strains of A background. Occasionally, the PFC of low responder mice were indistinguishable from those of high responder strains. This could be caused by a successful V-C translocation also in strains on A background, provided that a lack of this type of translocation is the mechanisms for unresponsiveness.

The possibility that mice on A background do not express Ig receptors, because they have been tolerized by exposure to bacterial products containing α-1-6 is highly unlikely. Thus, we have previously shown (19) that immunological tolerance to an epitope on Dx only affects a subpopulation of B cells with Ig receptors for the epitope. This subpopulation is characterized by having PBA receptors for Dx as well as Ig receptors for the epitope. Thus, tolerance is not due to clonal deletion and many specific B cells in tolerant mice can be activated by different PBA's to synthesize antibodies against the tolerogen. Similarly, we have shown (unpublished observations) that lymphocytes from Dx tolerant after treatment with dextranase can be activated by LPS, but not with Dx to the synthesis of antibodies against the tolerogen. Finally, Dxtolerant mice injected with dextranase can make specific high affinity antibodies to Dx after immunization with dextran coupled to a variety of TD and TI carriers, but never after immunization with Dx alone. These findings show that only the B cells which have both Ig and PBA receptors for Dx are irreversibly tolerized, other specific B cells remain unaffected and can be activated by immunization with dx coupled to other carriers. Since we have performed analogous experiments here (Tables III and V) and failed to activate anti-Dx antibodies in A.CA mice, the possibility that A.CA mice are nonresponders because they are tolerant can be ruled out.

A detailed study on the genetics of unresponsiveness to the α-1-6 epitope will be given elsewhere, but it is clear that the genes responsible are not linked to H-2 region since A, A.CA, A.SW, A.TH, and A.TL all have differences in different parts of the H-2 complex including the I region and they are all low responders. These strains have the same genetic background except H-2 indicating that the genes are not present in the major histocompatibility complex.

Summary

The immune response of mice to the α-1-6 epitope of dextran (Dx) B512 was found to be under genetic control. The congenic mouse strains A, A.CA, A.SW, A.TH, and A.TL exhibited a specific defect in their response to α-1-6. Also strain CBA/N was unresponsive to α-1-6, but the mechanism of unresponsiveness was found to be different.

Unresponsiveness to α-1-6 in congenic A strains was not due to suppressor cells. Although these strains failed to respond to the α-1-6 epitope, they responded strongly to the hapten Fluorescein isothiocyanate (FITC) conjugated to Dx, indicating that the Dx can function as an efficient carrier in these strains. Dx was a potent polyclonal B-cell activator in congenic A strains as well as in high responder strains. Polyclonally-activating concentrations of
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Lipopolysaccharide (LPS) failed to induce the synthesis of anti-α-1-6 antibodies in congenic A strains, although antibodies of all other specificities studied were produced. However, in high responder strains, LPS induced the synthesis of anti-α-1-6 antibodies. It was concluded that congenic A strains do not express V genes coding for antibodies against α-1-6.

In contrast, strain CBA/N failed to respond to both the α-1-6 and FITC epitope on Dx, whereas they could respond to FITC conjugated to horse erythrocytes. Dx induced a very small, if any, polyclonal antibody response in B cells from CBA/N mice or male CBA/N × DBA hybrids, whereas Dx was a very potent polyclonal B-cell activator in female hybrids. It is concluded that CBA/N mice are nonresponders to Dx or haptenated Dx, because the cell population that can respond to the polyclonal B-cell activating properties of Dx is severely depleted.

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