

Major Histocompatibility Complex Restriction of T-Cell Suppression of Immune Response to Mycobacteria¹

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The pathogenesis of T-cell nonresponsiveness in mycobacterial diseases such as leprosy is not clear. Two distinctly different mechanisms, namely, a genetic defect in antigen presentation⁽⁹⁾ and selective generation of suppression as a function of the route of immunization⁽¹¹⁾, have been proposed to explain the nonresponder state. The latter investigators⁽¹¹⁾ employed the intraperitoneal (i.p.) route of immunization of mice to show that suppressors were generated. Employing the same animal model, we reported that the killed vaccines of rapid-growing mycobacteria differed from those of slow growers in their immunogenicity to mice by the i.p. route, the former being non-immunogenic⁽¹³⁾. Failure of rapid growers to mount an immune response by the i.p. route could be due to two distinct mechanisms acting in isolation or combination, i.e., a) antigen presentation defect in the macrophage/antigen-presenting cell linked to major histocompatibility complex (MHC) class II or Ia antigens, and b) generation of suppressors.

In our earlier publications^(12, 14), we presented evidence in support of both mechanisms in the pathogenesis of nonresponsiveness of mice to the rapid-growing mycobacteria by the i.p. immunization route. Antigen presentation by the peritoneal macrophages was shown to be poor⁽¹²⁾. Animals also generated Lyt-2+ T suppressors on i.p. immunization⁽¹⁴⁾. In light of the poor antigen presentation by peritoneal

macrophages, it was surprising that immunization with the same organisms by the same route also triggered a T-suppressor (Ts) response which itself has to depend upon efficient antigen presentation. The paradox can be resolved if one postulates the triggering requirements, such as MHC restriction and cofactors of T-cell stimulation, to be different for Ts and for T cells responsible for help (Th) and delayed-type hypersensitivity (DTH).

In this publication we present evidence to show that the Ts response of the i.p. immunized mice is I-E restricted; whereas the Th response is I-A restricted. These data appear to support a role for both the so-called "immune response" (Ir) and "immune suppressor" (Is) genes in the pathogenesis of the nonresponder state, as has been proposed by Ottenhoff and de Vries⁽⁹⁾.

MATERIALS AND METHODS

Organism. *Mycobacterium vaccae* was kindly supplied by Dr. J. L. Stanford of Middlesex Hospital, London.

Animals. Four-to-six-week-old, BALB/c (H-2^d), C57BL/6 (H-2^b), C3H (H-2^k), and CBA (H-2^k) mice, obtained from the National Institutes of Health, Bethesda, Maryland, U.S.A. and maintained at the Cancer Research Institute, Bombay, India, were used in the study. The (BALB/c × C57BL/6) F₁ mice were bred in our animal house.

Preparation of sonic extract. A 1-week-old culture of rapid-growing *M. vaccae* was killed by 2.4 Mrad gamma radiation in a ⁶⁰Co source as described earlier⁽¹³⁾. The killed cells were washed with phosphate buffered saline (PBS), disrupted in a sonicator (Branson Sonifier, B-30), and spun at 50,000 × g × 1 hr. The supernatant, i.e., the sonic extract, was employed as "test antigen." The protein content of the test antigen was estimated as described earlier⁽¹³⁾.

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Immunization. A 1-week-old culture of *M. vaccae* was killed by irradiation, and the bacterial cells were harvested and washed in PBS (pH 7.2). The washed bacterial cells were pelleted by centrifugation at $2200 \times g \times 1$ hr. The supernatant was discarded, and the washed cell pellet was suspended in PBS to give 1% (v/v $\approx 10^9$ bacilli/ml) suspension. The required number of bacilli were suspended in 0.5 ml for i.p. immunization and 0.05 ml for intradermal (i.d.) immunization on the back at two or three sites.

DTH test. The delayed-type hypersensitivity test (DTH) was done as described earlier (¹³). Sonicate containing 50 μ g protein in 0.03 ml volume was injected into the hind foot pad and measurements of foot pad thickness were taken before, 24 hr, and 48 hr after injection. The sonicate was also tested in nonimmune mice as controls. The results were expressed as corrected foot pad enlargement (CPE), which is the difference between the foot pad enlargements caused by the test sonicate in the immunized and control mice simultaneously tested. A statistical Student's *t* test was done on CPE values by comparing with zero.

Lymphoproliferation test. A lymphoproliferation test was carried out using standard microculture techniques (⁸). Briefly, each well of a microtiter plate (Nunc, Denmark) received 2×10^5 primed spleen cells in 0.2 ml culture medium (RPMI 1640) containing 2.0 mM glutamine, 10% fetal calf serum, 1% antibiotic antimycotic (all from GIBCO, Grand Island, New York, U.S.A.), and 10 μ g/ml of the *M. vaccae* sonicate. Cultures were pulsed 120 hr later with 0.5 μ Ci of ³H-labeled thymidine for a period of 16 hr, harvested, and the incorporation of the label assayed and expressed as counts per minute (cpm). All tests were run in quadruplicate. When required, comparison of the cpm values was done by Student's *t* test.

Depletion of T cells, DTH effectors, and Ts. Depletion of T cells, DTH effectors, and Ts from the lymphoid cell suspensions was done by treatment with monoclonal anti-Thy-1.2, anti-L3T4, and anti-Lyt-2.2 antibodies and complement as described earlier (⁶). The monoclonal antibodies were kindly provided by Dr. C. S. Henney, Immunex Corporation, Carson City, Nevada, U.S.A.

Antibody to I-A^{bdq}, E^{dk}. Supernatants of the cultures of the rat IgG2b clone of the above anti-mouse anti-MHC class II specificity (²) were pooled. Antibody was precipitated with 33% (NH₄)₂ SO₄, dissolved in PBS, and dialyzed extensively. The protein concentration was adjusted as desired by concentration or dilution.

RESULTS

DTH responses of C57BL/6, BALB/c, and F₁ mice. The C57BL/6 (H-2^b), the BALB/c (H-2^d), and the (C57BL/6 \times BALB/c) F₁ mice were immunized with 10⁸ killed *M. vaccae* by the i.p. and i.d. routes, and their DTH responses to the sonicate were tested 1 week later. The i.d. route of immunization generated a DTH response in all three strains. However, after i.p. immunization it was observed that only the C57BL/6 mice were successful in generating DTH responsiveness; the BALB/c and the F₁ mice behaved as nonresponders (Table 1).

Immune suppression in C57BL/6, BALB/c, and F₁ mice. In order to investigate if i.p. immunization of the above three strains of mice with rapid-growing mycobacterium generates suppression, the following two protocols described in detail in our earlier publication (¹⁴) were employed.

The i.p. nonresponder BALB/c and the F₁ mice were given 10⁸ killed *M. vaccae* by the i.p. route as before. One week later they were given a second immunization of 10⁸ *M. vaccae* by the i.d. route. Intraperitoneal immunization 1 week prior to i.d. immunization completely abrogated the ability of the two strains of mice to respond to i.d. immunization (Table 1).

In order to investigate the characteristics of cells responsible for suppression in BALB/c and F₁ mice, the mice were immunized by the i.p. route as before. One week later their spleen cells were harvested and 4.0×10^7 cells were transferred to syngeneic recipients. The latter were then challenged by i.d. immunization. Mice receiving i.p.-primed spleen cells failed to mount an immune response to the i.d. immunization with *M. vaccae*, indicating generation of suppressor cells by i.p. priming. The ability of i.p.-primed spleen cells to adoptively transfer suppression could be completely abrogated by removal of cells having Thy-1

TABLE 1. Comparison of DTH responses of H-2^b, H-2^d, and (H-2^b × H-2^d) F₁ mice immunized with *M. vaccae* by different routes.^a

| Sr. no. | Mouse strain | H-2 haplotype | A | B | C | D |
|---------|----------------------------------|-------------------------------------|--------------------|-----------------------------|--------------------|-----------------------------|
| | | | Immunization route | CPE (mm) ± S.E. after 48 hr | Immunization route | CPE (mm) ± S.E. after 48 hr |
| 1 | C57BL/6 | H-2 ^b | i.p. | 0.26 ± 0.01 | — | — |
| 2 | C57BL/6 | H-2 ^b | i.d. | 0.28 ± 0.01 | — | — |
| 3 | BALB/c | H-2 ^d | i.p. | 0.08 ± 0.03 | i.d. | 0.02 ± 0.01 |
| 4 | BALB/c | H-2 ^d | i.d. | 0.36 ± 0.01 | — | — |
| 5 | (C57BL/6 × BALB/c)F ₁ | H-2 ^b × H-2 ^d | i.p. | 0.08 ± 0.01 | i.d. | 0.06 ± 0.01 |
| 6 | (C57BL/6 × BALB/c)F ₁ | H-2 ^b × H-2 ^d | i.d. | 0.29 ± 0.01 | — | — |

^a Mice were immunized (10 per group) with 10⁸ killed cells of *M. vaccae* either by i.p. or i.d. route (A) and 1 week later their DTH responses against *M. vaccae* sonicate were tested and reported as corrected foot pad enlargement (CPE) (B). The i.p. immunized mice which did not respond received a second immunization with 10⁸ *M. vaccae* by the i.d. route (C), and 1 week later their DTH responses were tested (D). CPE differences in 3 and 4 and 5 and 6 are statistically significant, *p* < 0.01.

and Lyt-2 markers by anti-Thy-1.2 and anti-Lyt-2.2 antibody and complement, indicating that the suppressors were in fact Ts cells. In Table 2, the data on F₁ mice are presented. The BALB/c mice also showed similar behavior (data not shown here; reported earlier¹⁴).

The experimental protocol of prior i.p. immunization suppressing the subsequent i.d. immunization could not be employed for testing the ability of C57BL/6 mice to generate suppression because they were responders to *M. vaccae* by the i.p. route. Hence, a different protocol (described earlier¹⁴) was employed. C57BL/6 mice were immunized i.p. with 10⁸ *M. vaccae*. One

week later their spleen cells were harvested, and the DTH effectors were eliminated with anti-L3T4 antiserum and complement treatment. Spleen cells (4 × 10⁷) depleted of cells with the L3T4 marker were transferred to syngeneic recipients which were then immunized i.d. with 10⁸ *M. vaccae*, and their DTH responses were tested 1 week later. The DTH response of the recipients was of the same order as that of the control mice simultaneously immunized by the i.d. route and tested; no suppression was observed (Table 3).

From these experimental data, it could be concluded that i.p. immunization with *M. vaccae* generated T-cell-mediated suppres-

TABLE 2. Generation of T-cell-mediated suppression after immunization of BALB/c and F₁ mice with *M. vaccae* by the i.p. route.

| Sr. no. | Mouse strain | Nature of cells received before i.d. immunization ^a | CPE (mm) ± S.E. after 48 hr ^b |
|---------|----------------|---|--|
| 1 | BALB/c | From nonimmune BALB/c | 0.28 ± 0.01 |
| 2 | BALB/c | From BALB/c primed i.p. with 10 ⁸ <i>M. vaccae</i> | 0.06 ± 0.01 |
| 3 | F ₁ | From nonimmune F ₁ | 0.23 ± 0.01 |
| 4 | F ₁ | From F ₁ primed i.p. with 10 ⁸ <i>M. vaccae</i> | 0.02 ± 0.01 |
| 5 | F ₁ | Anti-L3T4 + C-treated F ₁ cells primed i.p. with 10 ⁸ <i>M. vaccae</i> | 0.03 ± 0.01 |
| 6 | F ₁ | Anti-Lyt-2 + C-treated F ₁ cells primed i.p. with 10 ⁸ <i>M. vaccae</i> | 0.26 ± 0.02 |
| 7 | F ₁ | Anti-Thy-1.2 + C-treated F ₁ cells primed i.p. with 10 ⁸ <i>M. vaccae</i> | 0.24 ± 0.01 |

^a BALB/c and (C57BL/6 × BALB/c)F₁ mice were immunized with 10⁸ killed *M. vaccae* by the i.p. route. One week later the spleen cells were harvested and transferred to syngeneic recipients (10 per group) as such or after treatment with one of the antibodies shown plus complement to deplete the cells carrying the respective marker. Recipients were given i.d. immunization with 10⁸ *M. vaccae* and their DTH tested 1 week later.

^b CPE = corrected foot pad enlargement. CPE differences between 1 and 2 and between 3 and 4 and 5 are statistically significant, *p* < 0.01.

TABLE 3. Test for generation of T-cell-mediated suppression in C57BL/6 mice after i.p. immunization with *M. vaccae*.

| Sr. no. | Source of L3T4 depleted spleen cells ^a | CPE (mm) ± S.E. after 48 hr ^b |
|---------|--|--|
| 1 | Nonimmune C57BL/6 | 0.26 ± 0.06 |
| 2 | C57BL/6 mice primed i.p. with 10 ⁸ <i>M. vaccae</i> | 0.26 ± 0.06 |

^a Ten C57BL/6 mice were immunized with 10⁸ killed *M. vaccae* by the i.p. route. One week later the spleen cells were harvested, treated with anti-L3T4 antibody and complement. The surviving cells were pooled and 4 × 10⁷ such cells were transferred to 10 syngeneic recipients that were immunized with 10⁸ *M. vaccae* by the i.d. route. DTH responses were tested 1 week later.

^b CPE = corrected foot pad enlargement.

sion in BALB/c and F₁ mice but not in C57BL/6 mice.

MHC restriction of suppression. The F₁ mice were immunized i.p. with 10⁸ *M. vaccae*. One week later their spleen cells were harvested and transferred to syngeneic F₁, parental BALB/c mice and the C57BL/6 mice (4 × 10⁷ cells/mouse). The recipients were then immunized i.d. with 10⁸ *M. vaccae*, and their DTH responses were tested 1 week later. The C57BL/6 mice showed a DTH response comparable to that of the control mice simultaneously immunized. In contrast, the BALB/c and the F₁ mice failed to generate a DTH response (Table 4).

The MHC restriction of suppression by the F₁ cells was also investigated in an *in vitro* lymphoproliferative response. C57BL/6 and BALB/c mice were immunized i.d., and the F₁ mice were immunized i.p. with killed 10⁸ *M. vaccae*: 2 × 10⁵ i.d.-primed parental spleen cells were cocultured with 5 × 10⁴ splenic-T cells of i.p.-primed F₁ mice, and a 6-day lymphoproliferative assay against *M. vaccae* sonicate was performed. The i.p.-primed F₁ splenic-T cells could suppress the proliferative response of the BALB/c (50% reduction in cpm); whereas the response of the C57BL/6 mice was unaffected (Fig. 1).

From these data it could be concluded that the suppressor-T cells of the F₁ mice could induce suppression in the H-2^d but not in the H-2^b strain. Since the latter do not express I-E coded MHC class II anti-

TABLE 4. DTH suppression in parental H-2^b and H-2^d mouse strains by suppressors generated in F₁ mice after i.p. immunization^a with *M. vaccae*.

| Sr. no. | No. F ₁ sup-pres-sors ^b | Recip-ients ^c | Haplo-type | CPE (mm) ± S.E. after 48 hr ^d |
|---------|---|--------------------------|----------------------------------|--|
| 1 | 4 × 10 ⁷ | C57BL/6 | H-2 ^b | 0.28 ± 0.01 |
| 2 | | C57BL/6 | H-2 ^b | 0.23 ± 0.01 |
| 3 | 4 × 10 ⁷ | BALB/c | H-2 ^d | 0.03 ± 0.01 |
| 4 | | BALB/c | H-2 ^d | 0.27 ± 0.01 |
| 5 | 4 × 10 ⁷ | F ₁ | (H-2 ^b ^d) | 0.02 ± 0.01 |
| 6 | | F ₁ | (H-2 ^b ^d) | 0.23 ± 0.01 |

^a Twenty-five F₁ mice were immunized by the i.p. route with 10⁸ killed *M. vaccae*. One week later their DTH responses to *M. vaccae* sonicate were tested and the absence confirmed. Mice were then sacrificed and spleen cells were harvested and pooled: 4 × 10⁷ spleen cells (^b) were transferred i.v. into either C57BL/6 or BALB/c parental strains (^c) or the syngeneic F₁ strain (10 per group) which were then challenged by i.d. immunization with 10⁸ *M. vaccae*. Their DTH responses to *M. vaccae* sonicate were tested 1 week later.

^d CPE differences between 3 and 4 and between 5 and 6 are statistically significant, p < 0.01.

gens, the above data support a conclusion that the suppression was I-E restricted.

Effect of anti-I-A^{bdq}, E^{dk} antibody. The BALB/c (H-2^d) mice were immunized i.d. and the C3H and CBA (H-2^k) mice were immunized i.p. with 10⁸ killed *M. vaccae*. The DTH responses of the C3H and CBA mice to *M. vaccae* were very similar to those of the BALB/c mice, i.e., the organism was immunogenic by the i.d. route and non-immunogenic by the i.p. route. Further i.p. immunization generated T-cell-mediated suppression (data not shown). One week after immunization, the spleen cells were harvested and a lymphoproliferative assay against *M. vaccae* sonicate was performed in the presence or absence of monoclonal antibody of the specificity anti-I-A^{bdq}, E^{dk} at a final concentration of 100 µg/ml. Control cultures received normal mouse globulin at the same concentration. The antibody depressed the proliferative response of i.d.-primed spleen cells of BALB/c mice (40% reduction in cpm); whereas it enhanced the response of i.p.-primed spleen cells of the C3H and CBA mice (2.5- to 3-fold increase in cpm; Fig. 2).

This antibody is reactive with both I-A and I-E glycoproteins of BALB/c (H-2^d);

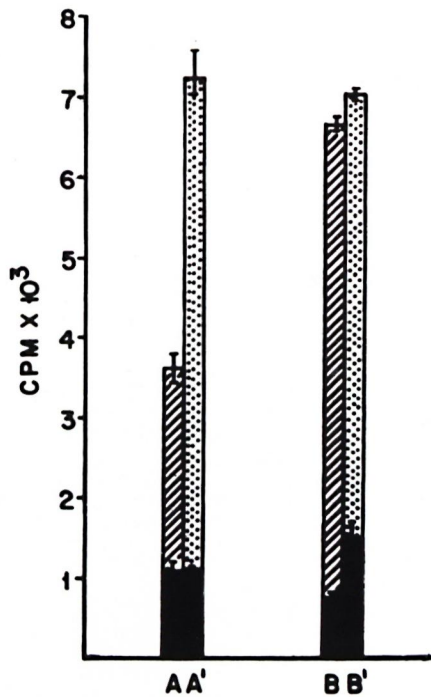


FIG. 1. *In vitro* suppression of parental lymphoproliferative response by suppressors generated in F_1 mice by i.p. immunization with *M. vaccae*. BALB/c and C57BL/6 mice were immunized i.d. and (C57BL/6 \times BALB/c) F_1 mice were immunized i.p. with 10^8 killed cells of *M. vaccae*; 2×10^5 splenic cells of i.d.-primed parental strains were cocultured with either 5×10^4 splenic-T cells of i.p.-primed F_1 mice or 5×10^4 splenic-T cells from nonimmune mice, and a standard 6-day LTT performed with $10 \mu\text{g/ml}$ *M. vaccae* sonicate. \square = A and B show proliferative responses of i.d.-primed splenic cells from BALB/c or C57BL/6 mice, respectively, in the presence of 5×10^4 splenic-T cells from i.p.-primed F_1 mice; \square = A' and B' show proliferative responses of i.d.-primed splenic cells from BALB/c or C57BL/6 mice, respectively, in the presence of 5×10^4 splenic-T cells from nonprimed F_1 mice; \blacksquare = proliferation in the absence of antigen. Difference between A and A' is statistically significant, $p < 0.01$.

whereas in C3H and CBA (H-2^k), it is reactive with only I-E (²). Hence, it could be concluded that the antibody suppressed the response of i.d.-primed splenic cells if the antibody recognized the I-A specificities, and enhanced the proliferative response if it recognized the I-E specificities.

Blockade of *in vitro* T-cell-mediated suppression by anti-I-A^{bdq}, E^{dk} antibody. CBA and C3H mice were immunized i.p. and i.d. with 10^8 *M. vaccae*; 2×10^5 i.d.-primed

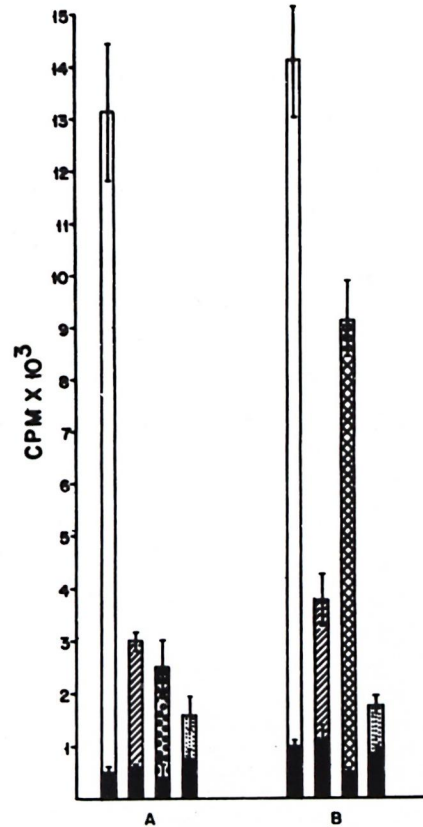


FIG. 2. Differential effect of anti-Ia (IA^{bdq}, IE^{dk}) antibody on *in vitro* proliferation of i.p. and i.d. *M. vaccae*-primed splenic cells of H-2^a and H-2^k haplotypes. Proliferation of 2×10^5 splenic cells of BALB/c (A) and C3H (B) mice primed i.p. or i.d. with *M. vaccae*, in the presence or absence of anti-Ia (IA^{bdq}, IE^{dk}) antibody. \blacksquare = Proliferation in absence of antigen; \square = proliferation of i.d.-primed splenic cells with antigen, without antibody; \square = proliferation of i.p.-primed splenic cells with antigen, without antibody; \square = proliferation of i.d.-primed splenic cells with antigen and anti-Ia antibody; \square = proliferation of i.p.-primed splenic cells with antigen and anti-Ia antibody; \square = nonprimed splenic cells and antigen. Differences in proliferative responses of i.p.- and i.d.-primed splenic cells (\square and \square), of i.d.-primed splenic cells of BALB/c with and without anti-Ia antibody (\square and \square), and of i.p.-primed splenic cells of C3H mice with and without anti-Ia antibody (\square and \square) are statistically significant, $p < 0.01$.

splenic cells were cocultured with 5×10^4 i.p.-primed syngeneic splenic-T cells with or without anti-I-A^{bdq}, E^{dk} antibody at a final concentration of $100 \mu\text{g/ml}$. The control cultures received normal mouse immunoglobulin at the same concentration. A standard 6-day lymphoproliferative test was

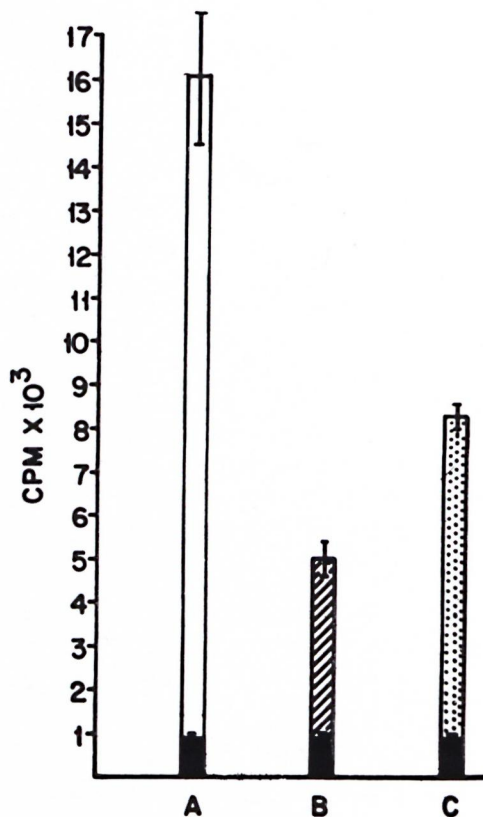


FIG. 3. Blockade of Ts-induced *in vitro* suppression of lymphoproliferative response by anti-I-E antibody. C3H mice were primed i.d. and i.p. with *M. vaccae*. □ = Proliferation of 2×10^5 i.d.-primed spleen cells in presence of 5×10^4 syngeneic normal spleen cells and antigen; ▨ = proliferation of 2×10^5 i.d.-primed spleen cells in the presence of 5×10^4 syngeneic i.p.-primed spleen cells and antigen; ▩ = proliferation of 2×10^5 i.d.-primed spleen cells in the presence of 5×10^4 syngeneic i.p.-primed spleen cells, antigen, and anti-Ia antiserum; ■ = proliferation of different cells without antigen. Differences between A and B and between B and C are statistically significant, $p < 0.01$.

performed against *M. vaccae* sonicate. The proliferative response of i.d.-primed cells was suppressed by splenic-T cells of i.p.-primed mice (60% reduction in cpm). When the above antibody was added to the culture, suppression was significantly blocked (Fig. 3).

DISCUSSION

In our previous publication (13) it was reported that the killed vaccines of rapid-growing mycobacteria differed from those of slow growers in their immunogenicity to

mice by the i.p. route, the rapid growers being nonimmunogenic. Two main factors which influence immunogenicity, namely, antigen presentation and generation of T-cell-mediated suppression, were investigated (12, 14).

So far as antigen presentation is concerned, the peritoneal macrophages behaved discriminately for slow- and rapid-growing mycobacteria. Whereas the slow-grower antigens were presented efficiently, rapid-grower antigens were not (12). In contrast, both groups of mycobacteria were observed to generate T-cell-mediated suppression (14). The resultant outcome of these two factors affected i.p. immunogenicity of the two groups of mycobacteria differently, the slow growers being immunogenic and the rapid growers, nonimmunogenic (13).

This prompted us to investigate further the relative importance of antigen presentation vis-a-vis suppression in affecting the outcome of immunization in the experimental model. Since presentation of rapid-grower antigen by the peritoneal macrophages was poor (12), it was surprising that the i.p. immunization with them generated T-suppression (14), because T-suppression is also dependent upon antigen presentation and MHC restriction. Hence, the MHC restriction requirement of T suppression was investigated.

Unlike the BALB/c and several other inbred mice strains and the outbred Swiss white mice, C57BL/6 mice could be immunized to rapid growers by the i.p. route. The efficiency of presentation of *M. vaccae* sonicate by the peritoneal cells of the C57BL/6 mice was not significantly different from that of the BALB/c mice (data not shown). Hence, it was logical to conclude that the difference in behavior of the C57BL/6 and the BALB/c mice to i.p. immunization with a rapid-growing mycobacteria such as *M. vaccae* was not due to a difference in the efficiency of antigen presentation by the peritoneal cells of the two respective mouse strains. The difference was apparently due to nongeneration of T-cell-mediated suppression by the C57BL/6 mice. Further, based on the following evidence, it could be concluded that the T suppression generated by i.p. immunization was I-E re-

stricted; whereas the T-helper response was I-A restricted.

a) The C57BL/6 mice which failed to generate suppression are I-E deficient (⁷). b) The (C57BL/6 × BALB/c) F₁ mice behaved like BALB/c, i.e., they generated T suppression. c) The F₁ T suppressors could suppress the immune response in parental BALB/c (H-2^d) but not in the C57BL/6 (H-2^b) mice. Since the F₁ mice are expected to express the I-E of the BALB/c (H-2^d) haplotype only, it could be concluded that the F₁ suppressors were restricted by the I-E of H-2^d specificity. d) The anti-I-A antibody could block the lymphoproliferative response of i.d.-primed spleen cells; whereas the anti-I-E antibody enhanced the proliferative response of the i.p.-primed spleen cells. e) The suppression of *in vitro* proliferative responses of i.d.-primed spleen cells by the i.p.-primed suppressors could be blocked by the anti-I-E antibody. Similar observations regarding I-E restriction of T-suppression have been reported for other antigens, such as LDH-β (¹), and parasitic antigens of *Trichinella spiralis* and *Nippostrongylus dubius* (¹⁶).

These data support a major role for antigen presentation and MHC restriction in the immunogenicity of mycobacteria. Antigen presentation for both Th/DTH and Ts responses seems to play a role in the outcome of immunization. Since in this model two responses are regulated by different MHC class II locus glycoproteins, the outcome of immunization by a given route would be dependent upon their interactions. When the Th presentation was efficient, as with slow growers (¹²), despite suppression, i.p. immunization succeeded in generating DTH responsiveness (¹⁴). On the other hand, when the Th presentation was poor, as with rapid growers (¹²), the outcome of immunization depended upon suppression. When suppression was absent, as with C57BL/6 mice (due to lack of I-E expression), i.p. immunization with *M. vaccae* generated DTH responsiveness, and when suppression was present, as with BALB/c mice, the i.p. immunization failed.

Several studies linking HLA and nonresponder states in leprosy have also suggested two distinctly different pathogenic mechanisms. In family and population studies (^{4, 5, 9, 10, 15}), a significant deficit in HLA-class

TABLE 5. Spectrum of responsiveness resulting from interactions of Ir- and Is-mediated regulations.

| Presentation | T-cell-mediated suppression | Immune response |
|--------------|-----------------------------|-----------------|
| Efficient | Absent | ++++ |
| Efficient | Present | ++ to +++ |
| Inefficient | Absent | + to ++ |
| Inefficient | Present | Absent |

D phenotype, such as DR-3, was observed among BL-LL leprosy patients. In contrast, both family and population studies have shown preferential inheritance of DQw1 by BL-LL cases (^{3, 9}). The pathology in the former situation can be visualized to be due to an antigen-presentation defect. In the latter situation, the most likely pathology would be selective generation of T-cell-mediated suppression induced by the particular HLA-class II glycoproteins. Thus, the data seem to suggest a role for both the so-called immune response (Ir) and immune suppressor (Is) genes. The spectrum of responsiveness seen in leprosy may also reflect the interactions of Ir and Is gene-mediated regulation as shown in Table 5.

SUMMARY

In earlier work, intraperitoneal (i.p.) immunization with *Mycobacterium vaccae* was shown to generate a T-suppressor (Ts) response but intradermal (i.d.) immunization did not. We have now studied the major histocompatibility complex (MHC) restriction of this Ts response. The ability of C57BL/6 (H-2^b), BALB/c (H-2^d), and the (C57BL/6 × BALB/c) F₁ mice to generate suppression after i.p. immunization with 10⁸ killed *M. vaccae* was investigated. The BALB/c and the F₁ mice generated suppression, but the C57BL/6 mice failed to do so. The suppression could be ascribed to Lyt-2+, L3T4- antigen-specific T cells. The F₁ suppressors generated after i.p. immunization could suppress the generation of T-cell responses to i.d. immunization with *M. vaccae* in the parental BALB/c but not in the C57BL/6 mice. Monoclonal anti-I-A antibody could suppress the antigen-induced proliferative response of mice primed i.d. with *M. vaccae*. In contrast, monoclonal

anti-I-E antibody enhanced antigen-specific proliferation of spleen cells primed i.p. with *M. vaccae*. The suppressors generated by i.p. priming of mice with *M. vaccae* could also suppress the *in vitro* antigen-induced proliferative response of i.d.-primed spleen cells; the suppression could be blocked by anti-I-E antibody. Thus, the T-cell-mediated suppression in the above experimental model was I-E restricted. The inability of the C57BL/6 mice to generate suppression after i.p. immunization with *M. vaccae* was ascribed to the lack of I-E expression by mice of H-2^b strain.

RESUMEN

En un trabajo previo, se demostró que mientras que la inmunización intraperitoneal (i.p.) con *Mycobacterium vaccae* condujo a la generación de células T-supresoras (Ts), la inoculación intradérmica (i.d.) no lo hizo. Ahora hemos estudiado la restricción genética (complejo mayor de histocompatibilidad) de esta respuesta. Se investigó la capacidad de los ratones C57BL/6 (H-2^b), BALB/c (H-2^d) y de los F₁ (C57BL/6 × BALB/c), para generar supresión después de la inmunización i.p. con 10⁸ *M. vaccae* muertos. Los ratones BALB/c y los F₁ generaron supresión pero los C57BL/6 no lo hicieron. La supresión pudo adscribirse a células T Lyt2+, L3T4-, específicas para el antígeno. Las células supresoras F₁ generadas después de la inmunización i.p., suprimieron las respuestas de las células T a la inmunización i.d. con *M. vaccae* en los ratones BALB/c pero no en los C57BL/6 paternos. El anticuerpo monoclonal anti-IA pudo suprimir la respuesta proliferativa de los esplenocitos de ratones preestimulados i.d. con *M. vaccae*. En contraste, el anticuerpo monoclonal anti-I-E aumentó la proliferación inducida por antígeno de las células de bazo estimuladas i.p. con *M. vaccae*. Las células supresoras generadas por la preinoculación i.p. de los ratones con *M. vaccae*, también suprimieron *in vitro* la respuesta proliferativa inducida por antígeno de las células de bazo generadas por inmunización i.d.; la supresión pudo bloquearse por anticuerpo anti-I-E. Así, la supresión mediada por células T en este modelo experimental fue restringida por I-E. La incapacidad de los ratones C57BL/6 para generar supresión después de la inmunización i.p. con *M. vaccae*, se adscribió a la falta de expresión de I-E por las células de los ratones de la cepa H-2^b.

RÉSUMÉ

Au cours de travaux antérieurs, on a montré que l'immunisation intrapéritonéale par *Mycobacterium vaccae* engendrait une réponse des T supresseurs (Ts), mais l'immunisation intradermique ne le faisait pas. Nous avons à présent étudié la restriction du complexe majeur d'histocompatibilité (CMH) à cette réponse Ts. La capacité des souris C57BL/6 (H-2^b), BALB/c (H-

2^d), et (C57BL/6 × BALB/d) F₁ de produire une suppression après immunisation intra-péritonéale avec 10⁸ *M. vaccae* tués a été examinée. Les souris BALB/c et F₁ ont montré une suppression mais les souris C57BL/6 ne l'ont pas fait. La suppression pouvait être attribuée aux cellules T spécifiques pour les antigènes Lyt-2+ et L3T4-. Les supresseurs F₁ produits après immunisation intra-péritonéale pouvaient supprimer les réponses par les cellules T à l'immunisation intradermique avec *M. vaccae* chez la souche BALB/c mais pour les souris C57BL/6. Les anticorps monoclonaux anti-IA pouvaient supprimer la réponse proliférative induite par l'antigène des souris infectées en intra-dermique avec *M. vaccae*. Par contre, les anticorps monoclonaux anti-IE augmentaient la prolifération, spécifique pour l'antigène, des cellules spléniques après injection intra-péritonéale de *M. vaccae*. Les supresseurs produits par l'injection intra-péritonéale des *M. vaccae* à des souris pouvaient aussi supprimer *in vitro* la réponse proliférative de cellules spléniques à une injection intra-dermique de l'antigène; la suppression pouvait être bloquée par les anticorps anti-IE. Donc, la suppression due à la médiation des cellules T dans le modèle expérimental ci-dessus était restreinte par les I-E. L'incapacité des souris C57BL/6 de produire une suppression après immunisation intra-péritonéale avec *M. vaccae* était attribuée au manque d'expression des I-E par les souris de la souche H-2^b.

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