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Genetic Control of the Immune Responsiveness to *Streptococcus mutans* by the Major Histocompatibility Complex of the Rat (*RT1*)

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The lymph node cells from 11 strains of rats, differing in the genotype of the major histocompatibility complex of the rat (*RT1*), were examined on the basis of their proliferative response to the cell wall antigen of *Streptococcus mutans*. The 11 rat strains fell into three groups: high, intermediate, and low responders. To demonstrate the influence of the major histocompatibility complex on immune responsiveness to *S. mutans*, further experiments were performed using the *RT1*-congenic rat strains WKAH.1L(LEW), WKAH.1AV1(ACI), and WKAH.1J(LEJ), which differ only in the genotype of the *RT1* region. Although the background genes of each strain were of WKAH origin, WKAH.1L(LEW) and WKAH.1AV1(ACI) rats showed a low response whereas WKAH.1J(LEJ) rats showed a moderate response to the *S. mutans* cell wall antigen. The results indicate that the immune response is controlled by the class II gene(s) in *RT1*. Furthermore, the *RT1.D* locus products were shown to play an important role in the restriction molecule, since a monoclonal antibody, HOK7, directed to the *RT1.D^k* locus products reduced the proliferative response of lymph node cells.

The genetic control of immune responsiveness by the major histocompatibility complex (MHC) was first demonstrated by McDevitt and Chinitz (14). Since then, a large number of reports have been accumulated in mouse, human, and rat systems (1, 2, 4, 7, 9). The MHC of rats, which was designated as *RT1*, has been demonstrated to control immune responsiveness to some synthetic polypeptides such as TGAL (7) and to some natural antigens such as insulin (9).

Streptococcus mutans has been strongly implicated as a causative organism of dental caries (8). As reported by several laboratories, active development of host immunity to *S. mutans* by administration of whole cells or protein derivatives of this organism results in prevention of the induction of dental caries to a great extent (13, 15, 20). Since the administration of *S. mutans* as vaccination failed to prevent dental caries in thymectomized rats (5), it was obvious that T-cell response is important in eliminating *S. mutans* from experimental animals. Then the question was raised whether the immune response to *S. mutans* is controlled by the MHC or not. Sasazuki et al. reported that the responsiveness of T cells to particular streptococci was controlled by the MHC (*HLA*) in humans (19). It is possible that the immune responsiveness to *S. mutans* is also controlled by the MHC. There have been only a few reports on the involvement of MHC gene products in *S. mutans*-specific T-cell activation (10–12). Here we report the involvement of *RT1* products in the immune response to *S. mutans* in the rat system.

MATERIALS AND METHODS

Animals. Male rats, 3 to 6 months old, maintained at the Institute for Animal Experiments, Hokkaido University School of Medicine, were used in this study. *RT1*-congenic strains were produced and maintained in our animal facility.

Monoclonal antibodies. The production and characteristics of monoclonal antibodies HOK7 (anti-*RT1.D*), HOK12 (anti-*RT1.B*), and HOK33 (anti-*RT1.B*) were described previously (17). The subclasses of these monoclonal antibodies were immunoglobulin G2b (IgG2b), IgM, and IgG2b, respectively.

Preparation of SAg. *S. mutans* antigen (SAg) was prepared as follows. *S. mutans* was grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) for 24 h at 37°C. Cells were collected by centrifugation at 16,000 × *g* and were washed three times with distilled water. After destruction of the *S. mutans* cells with a cell homogenizer (cell homogenizer model MSK; B. Braun Apparatebau, Melsungen, Federal Republic of Germany) at 4,000 oscillations per min for 7 min, the supernatant was collected by centrifugation at 450 × *g* for 20 min. The insoluble fraction was collected by centrifugation at 20,000 × *g* for 60 min and was washed three times with distilled water.

This insoluble cell wall-rich fraction was suspended in distilled water and was digested with RNase and DNase. First, the fraction was digested with RNase (P-L Biochemicals, Inc., Milwaukee, Wis.) at a final concentration of 10 μg/ml at 37°C for 2 h, followed by three washes with phosphate-buffered saline. It was then digested with DNase (P-L Biochemicals) at a final concentration of 10 μg/ml at 37°C for 2 h, followed by three washings with phosphate-buffered saline.

After digestion with RNase and DNase, an acid extract was obtained by a method described elsewhere (19). The insoluble fraction, suspended in distilled water, was adjusted to pH 2.0 with 1 N HCl and was heated at 95°C for 10 min. After cooling to room temperature and neutralization with 1 N NaOH, the supernatant was collected by centrifugation at 20,000 × *g* for 30 min.

After being salted out with 33% saturated $(NH_4)_2SO_4$, the soluble fraction was collected by centrifugation at 10,000 × *g* for 30 min and was salted out again with 60% saturated $(NH_4)_2SO_4$. The sediment that was salted out with 60% saturated $(NH_4)_2SO_4$ was collected by centrifugation at 20,000 × *g* for 30 min, suspended in distilled water, and dialyzed against distilled water for 48 h at 4°C. The insoluble

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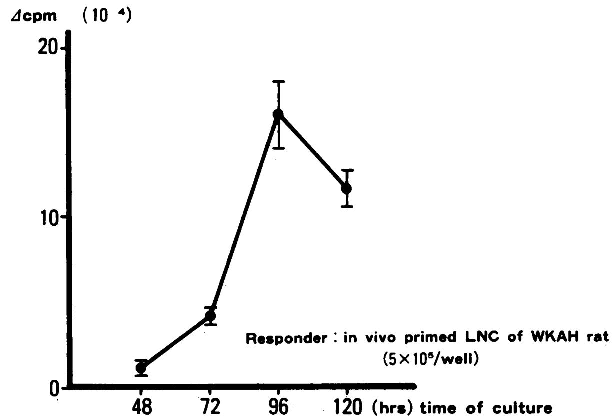

FIG. 1. Time course of in vitro SAg-induced proliferative response of lymph node cells. Lymph node cells from WKAH rats immunized with whole cells of *S. mutans* B13 (serotype d) were incubated in triplicate with 10 μg of SAg (serotype d) per well for various periods as indicated. The proliferative response was evaluated by [^{3}H]thymidine incorporation of the culture.

fraction was separated from the dialysate by centrifugation at 16,000 × *g* for 30 min, and the supernatant was lyophilized.

The resulting antigen (SAg) was dissolved in RPMI 1640 supplemented with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U of penicillin per ml, and 0.1 mg of streptomycin per ml and was sterilized by filtration through a 0.22-μm-pore-size filter (Sartorius, Tubingen, Federal Republic of Germany). This antigen preparation was frozen and stored at −80°C.

Immunization. *S. mutans* was grown in Trypticase soy broth for 24 h at 37°C and collected by centrifugation as described above, followed by fixation with 1% Formalin for 48 h at 4°C. The cells were collected by centrifugation, washed three times with distilled water, lyophilized, and then dissolved with phosphate-buffered saline to a final concentration of 2 mg/ml. When immunizing, the cell suspension was mixed with an equal volume of Freund incomplete adjuvant (Difco Laboratories, Detroit, Mich.) and 0.1 ml of emulsion was injected into the footpads of rats.

Proliferative response of lymph node cells to SAg. Fourteen days after immunization, draining lymph nodes were removed and washed and the lymph node cells were suspended in RPMI 1640 supplemented with 10% heat-inactivated Nu-Serum (Collaborative Research Inc., Lexington, Mass.), 25 mM HEPES, 0.05 mM 2-mercaptoethanol, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U of penicillin per ml, and 0.1 mg of streptomycin per ml. The lymph node cells (5×10^5 per well) in 0.2 ml of medium were cultured with or without SAg in 96-well flat-bottomed microtiter plates (Falcon 3072; Becton Dickinson and Co., Oxnard, Calif.) for 96 h at 37°C in a 5% CO_2 humidified atmosphere. [^{3}H]thymidine (1 μCi; New England Nuclear Corp., Boston, Mass.) was added to each well 8 h before harvest. The proliferative response was evaluated by the amount of [^{3}H]thymidine incorporation into DNA, which was measured by the standard liquid scintillation counting technique. Values were expressed as mean change in counts per minute (Δcpm) ± standard deviation of the triplicate culture: Δcpm = (cpm of the culture with antigen − cpm of the culture without antigen). In some experiments, monoclonal antibodies were added to the wells at the initiation of the culture. Percent inhibition was calculated as follows: % inhibition = 1 − (Δcpm in the presence of monoclonal antibody/Δcpm without monoclonal antibody) × 100.

RESULTS

Proliferative response of lymph node cells to *S. mutans* antigen. The proliferative response to *S. mutans* antigen (SAg) (serotype d) of lymph node cells obtained from WKAH rats immunized with whole cells of *S. mutans* B13 (serotype d) was examined at various times during culture. In this experiment, the maximum response was observed after 96 h of culture (Fig. 1). Figure 2 shows the proliferative response of lymph node cells stimulated in vitro with various concentrations of SAg. [^{3}H]thymidine incorporation increased, depending on the amount of SAg added. Since the application of more than 5 μg of SAg per well elicited a significant level of proliferation, the lymph node cells were stimulated with 5 to 10 μg of SAg per well and cultured for 96 h in the following experiments.

When animals were immunized with whole cells of *S. mutans* B13 (serotype d) or MT8148 (serotype c), the proliferation of the lymph node cells was observed if cells were stimulated in vitro with SAg of the relevant serotype (Table 1). Sham-immunized rats showed a good response to 0.5 μg of concanavalin A per well (224,223 cpm) but only a trace amount of response to 20 μg of SAg prepared from serotypes c and d per well (1,075 and 856 cpm, respectively). The results indicate that the response is highly specific, and they exclude the possibility that SAg induced nonspecific proliferation of lymph node cells.

Regulation of the response of lymph node cells to SAg by *RT1*. The lymph node cells obtained from 11 strains of rats were then examined for their proliferative response to SAg (serotype d) (Table 2). The proliferative response of lymph node cells of LEW, NIGIII, F344, and ACI rats was relatively low; that is, [^{3}H]thymidine incorporation by these cells was 5,916, 14,169, 8,486, and 13,911 cpm, respectively. On the other hand, the proliferative response of lymph node cells of SDJ rats was relatively high; that is, [^{3}H]thymidine incorporation by these cells was 154,961 cpm. Lymph node cells of LEJ, BUF, ALB, WKAH, W/Hok, and KYN rats showed moderate responses: [^{3}H]thymidine incorporation

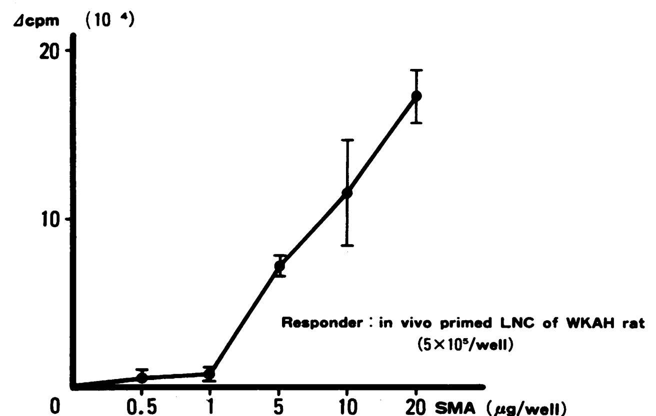

FIG. 2. Dose-dependent curve of in vitro SAg (SMA)-induced proliferative response of lymph node cells. Lymph node cells from WKAH rats immunized with whole cells of *S. mutans* B13 (serotype d) were incubated in triplicate with various amounts of SAg (serotype d) for 96 h. The proliferative response was evaluated by [^{3}H]thymidine incorporation of the culture.

TABLE 1. Proliferative response of lymph node cells to SAg derived from *S. mutans* serotypes c and d[a]

In vitro stimulation (μg/well)	Immunogen in vivo (Δcpm ± SD)	
	8148 (serotype c)	B13 (serotype d)
SAg (serotype c)		
10	160,857 ± 46,462	7,019 ± 2,166
20	194,947 ± 21,448	15,030 ± 1,712
SAg (serotype d)		
10	28,229 ± 5,708	135,984 ± 6,289
20	43,745 ± 4,060	139,819 ± 19,669

[a] WKAH rats were immunized with whole cells of *S. mutans* serotype c or d. The lymph node cells of immunized rats were cultured with SAg derived from *S. mutans* serotype c or d. Counts per minute without antigen were less than 1,500.

[b] Values are expressed as mean Δcpm ± standard deviation of the triplicate culture.

was 54,984, 35,117, 39,005, 48,301, 39,711, and 52,925 cpm, respectively. We regarded LEW, NIGIII, F344, and ACI rats as low responders, SDJ rats as high responders, and the remaining six strains as intermediate responders. [^{3}H]thymidine incorporation by intermediate or high responders was more than three times as much as that by low or intermediate responders, respectively. The difference of [^{3}H]thymidine incorporation among these three groups is statistically significant ($P < 0.01$).

To investigate the influence of *RT1* on the regulation of the response of lymph node cells to SAg, *RT1*-congenic rats were used in the next experiment (Table 3). [^{3}H]thymidine incorporation by lymph node cells of WKAH.1L(LEW) rats ($RT1^l$) and WKAH.1AV1(ACI) rats ($RT1^{avl}$) was 13,706 and 23,237 cpm, respectively. These two strains showed a significantly lower response than WKAH rats (Table 2). In contrast, [^{3}H]thymidine incorporation by lymph node cells of W/Hok.1U(SDJ) rats ($RT1^u$) was 129,550 cpm, which was significantly higher than that of W/Hok rats. These results clearly indicate that *RT1* controls the immune response to SAg. Since the WKAH.1J(LEJ) rats ($RT1.A^uB^bD^b$) showed a moderate response (61,374 cpm) whereas W/Hok.1U(SDJ) rats ($RT1.A^uB^uD^u$) showed a high response, it is suggested that *RT1.B* and/or *RT1.D* loci are responsible for the regulation of the immune response to SAg.

Demonstration of the restriction molecule involved in the proliferative response of lymph node cells to SAg. Three

TABLE 2. Strain difference of the proliferative response of lymph node cells to SAg (serotype d)[a]

RT1 haplotype	Strain (*n*)	[^{3}H]thymidine incorporation[b] (Δcpm ± SD)	Response pattern
l	LEW (10)	5,916 ± 3,406	Low
q	NIGIII (5)	14,169 ± 4,323	Low
lvl	F344 (3)	8,486 ± 1,389	Low
avl	ACI (5)	13,911 ± 7,568	Low
j	LEJ (5)	54,961 ± 14,850	Intermediate
b	BUF (3)	35,117 ± 5,674	Intermediate
b	ALB (3)	39,005 ± 7,991	Intermediate
k	WKAH (10)	48,301 ± 16,929	Intermediate
k	W/Hok (3)	39,711 ± 12,262	Intermediate
k	KYN (4)	52,925 ± 25,577	Intermediate
u	SDJ (4)	154,961 ± 109,577	High

[a] Lymph node cells from strains immunized with whole cells of *S. mutans* serotype d were stimulated with SAg (serotype d). [^{3}H]thymidine incorporation was measured as described in Materials and Methods.

[b] Values are expressed as mean Δcpm ± standard deviation of the triplicate culture.

TABLE 3. Association between responsiveness of lymph node cells and *RT1* haplotype[a]

Strain	*RT1* haplotype	[^{3}H]thymidine incorporation[b] (Δcpm ± SD)	Response pattern
LEW	*l*	7,915 ± 1,992	Low
ACI	*avl*	12,151 ± 2,999	Low
LEJ	*j*	69,243 ± 8,645	Intermediate
SDJ	*u*	136,350 ± 30,514	High
WKAH.1L(LEW)	*l*	13,706 ± 3,953	Low
WKAH.1AV1(ACI)	*avl*	23,237 ± 5,718	Low
WKAH.1J(LEJ)	*j*	61,374 ± 11,768	Intermediate
W/Hok.1U(SDJ)	*u*	129,550 ± 33,852	High

[a] The design of the experiment is the same as that shown in Table 2, footnote *a*.

[b] Values are expressed as mean Δcpm ± standard deviation of the triplicate culture.

monoclonal antibodies, HOK7, HOK12, and HOK33, directed to the *RT1* class II gene products were used to demonstrate the inhibitory effect of these monoclonal antibodies on the interaction between responding cells and antigen-presenting cells. Monoclonal antibodies at 2.5, 1.25, 0.5, or 0.05 μg of IgG or IgM per well were added at the initiation of the culture, and after 96 h of incubation [^{3}H]thymidine incorporation was measured. The addition of 2.5 μg of IgG per well of HOK7 (anti-$RT1.D^k$) resulted in 100% inhibition of the response (Fig. 3). The inhibition of the response by HOK7 was dose dependent. On the other hand, the response was only slightly affected by monoclonal antibodies HOK12 and HOK33. Table 4 shows the results of the same experiments using W/Hok and KYN rats. Similarly, only HOK7 significantly reduced the proliferative response in these experiments. These observations indicate that the SAg-specific immune response was restricted by *RT1.D* locus products.

DISCUSSION

Inbred or congenic rats were demonstrated to elicit an immune response to acid-extracted antigens of *S. mutans*

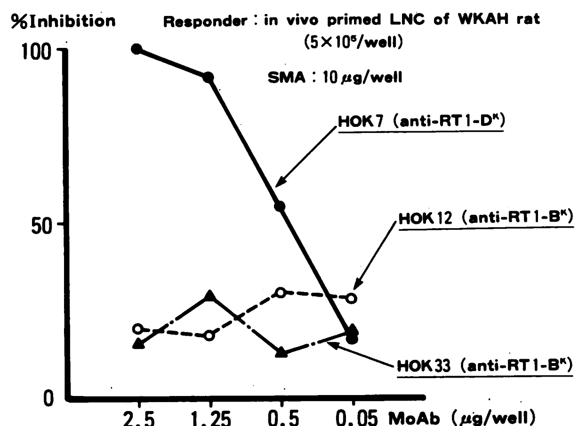

FIG. 3. Inhibitory effect of monoclonal antibodies on SAg (SMA)-induced proliferative response of lymph node cells. Lymph node cells from WKAH rats immunized with whole cells of *S. mutans* B13 (serotype d) were incubated in triplicate with 10 μg of SAg (serotype d) per well at 37°C for 96 h. Various amounts of monoclonal antibodies were added at the onset of the culture. The proliferative response was evaluated by [^{3}H]thymidine incorporation of the culture.

TABLE 4. Inhibitory effect of monoclonal antibodies on SAg-induced proliferative response of lymph node cells[a]

Monoclonal antibody	W/Hok		KYN	
	[^{3}H]thymidine incorporation[b] (Δcpm ± SD)	% Inhibition	[^{3}H]thymidine incorporation[b] (Δcpm ± SD)	% Inhibition
None	26,924 ± 4,011		83,362 ± 15,518	
HOK7	2,676 ± 3,448	90.1	7,353 ± 2,599	91.2
HOK12	29,657 ± 9,537	−10.2	70,963 ± 20,922	14.9
HOK33	28,127 ± 3,387	−4.5	72,742 ± 39,182	12.7

[a] Lymph node cells from strains immunized with whole cells of *S. mutans* B13 (serotype d) were stimulated with SAg (serotype d). Monoclonal antibodies (2.5 μg of IgG or IgM per well) were added at the onset of the culture. [^{3}H]thymidine incorporation was measured as described in Materials and Methods.

[b] Values are expressed as mean Δcpm ± standard deviation of the triplicate culture.

(SAg). This antigen preparation showed one strong peak and two weak peaks in a gel filtration assay. Only the former peak, distributed in a fraction of more than 500,000 daltons, elicited a strong immune response (data not shown). A dose of 5 to 10 μg per well, which was assumed to avoid a proliferative response to contaminating molecules, was chosen in the proliferation assay. Although whether the antigenic determinants were on the protein or the carbohydrate structure was not determined, the antigen preparation was revealed to elicit a serotype-specific proliferative response of lymph node cells (Table 1). The results suggested that the antigenic epitope is preserved after extensive acid extraction of SAg with heat, which might reflect the carbohydrate nature of the epitope. The proliferative response to irrelevant SAg could be due to a cross-reaction or, alternatively, to a response to molecules coexisting in the antigen preparation.

The proliferative response to SAg of lymph node cells obtained from 11 strains of rats differing in the *RT1* genotype was examined (Table 2). The amount of [^{3}H]thymidine incorporation by lymph node cells varied among the strains, ranging from 5,916 cpm (LEW rats) to 154,961 cpm (SDJ rats). We divided the rat strains into three groups by the degree of the proliferative response. LEW, NIGIII, F344, and ACI rats belonged to the low responder group; LEJ, BUF, ALB, WKAH, W/Hok, and KYN rats belonged to the intermediate responder group; and SDJ rats were considered to be high responders. Rats with the same *RT1* haplotype, for example WKAH, W/Hok, and KYN, which are typed as $RT1^k$, or BUF and ALB rats, which are typed as $RT1^b$, belonged to the same group of intermediate responders. It is noteworthy that LEW, NIGIII, and F344 rats, having the same class II haplotype, $RT1.B^lD^l$, belonged to the low responder group. These observations suggest that the *RT1.B* and/or *RT1.D* loci control the immune response to SAg. This was further confirmed by experiments using *RT1*-congenic rats. WKAH.1L(LEW), WKAH.1AV1(ACI), and WKAH.1J(LEJ) rats had the same background genes of WKAH origin and differed only in the *RT1* regions derived from LEW, ACI, and LEJ, respectively. The *RT1* region of W/Hok.1U(SDJ) was derived from SDJ, and the background genes were of W/Hok origin. Although WKAH and W/Hok rats were intermediate responders, introduction of the *RT1* regions from LEW or ACI (low-responder) rats into WKAH rats resulted in reducing the proliferative response to SAg (13,706 and 23,237 cpm, respectively, versus 48,301 cpm [WKAH]; Table 2). In contrast, introduction of the *RT1* region from LEJ (intermediate-responder) ($RT1.A^uB^bD^b$) or SDJ (high-responder) ($RT1.A^uB^uD^u$) rats into WKAH or W/Hok rats resulted in moderate or high proliferative response (61,374 and 129,550 cpm, respectively; Table 3). These observations clearly indicate that the *RT1* genes, specifically the *RT1.B* and/or *RT1.D* loci, play a major role in regulating the immune responsiveness to SAg.

It has been generally accepted that T cells are stimulated by foreign antigens in the context of the MHC class II gene products on the cell surface of antigen-presenting cells (18, 21). The *RT1* class II region is composed of *B* and *D* loci. Thus the SAg-specific response was considered to be restricted by either of these two loci. The response to SAg was reduced by a monoclonal antibody, HOK7 (Fig. 3), indicating that this response is restricted by *RT1.D* locus products. This is, to our knowledge, the first report of an *RT1.D*-restricted response to *S. mutans*. The functional differences of the *RT1.D* locus products among the strains may affect the T-cell-antigen-presenting cell interaction and consequent T-cell proliferative response to SAg.

S. mutans has been strongly implicated as a causative organism of dental caries (8). As reported by several laboratories, active development of host immunity to *S. mutans* prevents the induction of dental caries to a great extent (13, 15, 20). Here we report that the *RT1* region exerts an influence to a degree on the proliferative response of lymph node cells to SAg. It is possible that this may be responsible for a difference in the ability of host immunity to eliminate *S. mutans* from teeth. In fact, it was demonstrated that *H-2* (murine MHC) affected susceptibility to dental caries in mice (21). However, it is not clear whether *RT1* controls susceptibility to dental caries in rats. In humans, activity of helper T cells was revealed to be different between *DR4*- and *DRw6*-positive subjects (12), although de Vries et al. denied the association between *HLA* (human MHC) and dental caries (3). Since rats are widely utilized to investigate development and prevention of dental caries, further examinations on the association between *RT1* and susceptibility to dental caries are expected to elucidate the immunological factors of the host in the development of dental caries.

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LITERATURE CITED

1. **Ball, E. J., and P. Stastny.** 1984. Antigen-specific T-cell lines. II. A GAT-specific T-cell line restricted by a determinant carried by an HLA-DQ molecule. Immunogenetics **20:**547–564.
2. **Chan, M. M., W. B. Bias, S. H. Hsu, and D. A. Meyers.** 1984. Genetic control of major histocompatibility complex in man: poly(L-phenylalanine, L-glutamic acid)-poly(DL-alanine)--poly(L-lysine) and L-glutamic acid, L-alanine, L-tyrosine (60:30:10). Proc. Natl. Acad. Sci. USA **81:**3521–3525.
3. **de Vries, R. R. P., P. Zeylemaker, W. H. van Palenstein Helderman, and J. H. J. Huis in't Veld.** 1985. Lack of association between HLA-DR antigens and dental caries. Tissue Antigens **25:**173–174.
4. **Dorf, M. E.** 1981. Genetic control of immune responsiveness, p. 221–254. *In* M. E. Dorf (ed.), The role of the major histocompatibility complex in immunobiology. Garland STPM Press, New York.
5. **Ebersole, J. L., M. A. Taubman, and D. J. Smith.** 1982. Effect of neonatal thymectomy on dental caries in rats. Infect. Immun. **38:**1130–1136.
6. **Gill, T. J., III, D. V. Cramer, H. W. Kunz, and D. N. Misra.** 1983. Structure and function of the major histocompatibility complex of the rat. J. Immunogenet. **10:**261–273.

7. **Günther, E., E. Rüde, M. Meyer-Delius, and O. Stark.** 1973. Immune-response genes linked to the major histocompatibility system in the rat. Transplant. Proc. **5:**1467–1469.
8. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44:**331–384.
9. **Inomata, T., Y. Fujimoto, T. Natori, and M. Aizawa.** 1983. The genetic control of the immune response to insulins in the rat. Transplant. Proc. **15:**1593–1596.
10. **Lehner, T.** 1982. The relationship between human helper and suppressor factors to a streptococcal protein antigen. J. Immunol. **129:**1936–1940.
11. **Lehner, T.** 1983. Antigen-binding human T suppressor cells and their association with the HLA-DR locus. Eur. J. Immunol. **13:**370–378.
12. **Lehner, T., J. R. Lamb, K. L. Welsh, and R. J. Batchelor.** 1981. Association between HLA-DR antigens and helper cell activity in the control of dental caries. Nature (London) **292:**770–772.
13. **Lehner, T., M. W. Russell, J. Caldwell, and R. Smith.** 1981. Immunization with purified protein antigens from *Streptococcus mutans* against dental caries in rhesus monkeys. Infect. Immun. **34:**407–415.
14. **McDevitt, H. O., and A. Chinitz.** 1969. Genetic control of the antibody response: relationship between immune response and histocompatibility (H-2) type. Science **163:**1207–1209.
15. **Michalek, S. M., J. R. McGhee, and J. L. Babb.** 1978. Effective immunity to dental caries: dose-dependent studies of secretory immunity by oral administration of *Streptococcus mutans* to rats. Infect. Immun. **19:**217–224.
16. **Mizuno, K., T. Inomata, S. Tsuchimoto, Y. Matsuno, T. Niiyama, T. Natori, M. Aizawa, and D. Cramer.** 1985. The genetic control of the immune response to insulin in the rat. II. The low responsiveness in the WKAH rat is governed by suppressor T cells. Transplant. Proc. **17:**1837–1839.
17. **Natori, T., Y. Fujimoto, H. Ikeda, N. Ishikawa, H. Ishikura, Y. Matsuno, S. Tsuchimoto, K. Mizuno, T. Niiyama, D. Cramer, and M. Aizawa.** 1985. The RT1.B/D-region products defined by rat monoclonal alloantibodies. Immunogenetics **22:**511–516.
18. **Rosenthal, A. S., and E. M. Schevach.** 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. J. Exp. Med. **138:**1194–1212.
19. **Sasazuki, T., H. Kaneoka, Y. Nishimura, R. Kaneoka, M. Hayama, and H. Ohkuni.** 1985. An HLA-linked immune suppression gene in man. J. Exp. Med. **152:**297–313.
20. **Smith, D. J., M. A. Taubman, and J. L. Ebersole.** 1979. Effect of oral administration of glucosyltransferase antigens on experimental dental caries. Infect. Immun. **26:**82–89.
21. **Suzuki, H.** 1985. Caries susceptibility of mice of different strains infected with *Streptococcus mutans*. Jpn. J. Pedodont. **23:**198–203. (In Japanese.)