Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 1221–1224, February 1984
Immunology

Thymic T cells are driven to expand upon interaction with self-class II major histocompatibility complex gene products on accessory cells

(T-cell repertoire/major histocompatibility complex restriction/Ia molecule/interleukin 1)

KENNETH L. ROCK AND BARUJ BENACERRAF

Department of Pathology, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115

Contributed by Baruj Benacerraf, November 4, 1983

ABSTRACT **Murine thymocytes induce the monokine interleukin 1 upon *in vitro* coculture with a radioresistant Ia-bearing accessory cell [murine Ia molecule is a class II major histocompatibility complex (MHC) antigen]. The generation of interleukin 1 is critically dependent on the function of *I*-region gene products on accessory cells. The induced interleukin 1 appears to allow the activation and proliferation of self-MHC-specific thymocytes. Thus, in the absence of added exogenous factors, there is an Ia-dependent thymocyte proliferation. This selective activation of thymocytes is observed with both mature and immature thymic T cells. This *in vitro* response results in the selective amplification of developing T cells with self-MHC specificity and could be of importance to the *in vivo* commitment of T cells to MHC determinants that occurs in the thymus.**

The generation of functional mature T lymphocytes requires a thymus (1, 2). In this organ, striking T-cell proliferation and cell death are observed from which emerge functionally mature cells with a highly selected range of specificities (2). The T cells migrating from the thymus bear receptors that recognize nominal antigens in association with self-major histocompatibility complex (MHC) molecules (3, 4). This T-cell MHC specificity is determined by the allelic MHC gene products expressed in the thymus prior to a T-cell's experience with nominal antigen (4, 5). The mechanism underlying this commitment to self-MHC specificities is unknown.

It is generally accepted, nevertheless, that thymic specification involves the selective expansion of T cells with specificity for self-MHC molecules (6–8). Therefore, we investigated the signals required for the proliferation of developing T cells. We found that thymocytes recognize self-Ia molecules (*I*-region-associated murine class II MHC antigen) on accessory cells and are driven to expand in the presence of the monokine interleukin 1 (IL-1) (9). Thymocytes, relative to mature peripheral T cells, are uniquely reactive to these signals in the absence of foreign antigens. The existence of such a response in the thymus could be of importance in the commitment of T cells to MHC determinants that occurs in this organ. It was of interest, therefore, to analyze whether such a self-restricted expansion would occur spontaneously without added monokine. Such an analysis is the subject of this report.

MATERIALS AND METHODS

Mice. C3H/Hej and C3H/Fej mice were purchased from The Jackson Laboratory.

Monoclonal Antibodies (Mabs). MAb-containing culture supernatant was obtained from the hybridomas MKD6 (10) (anti-I-A^d), 10.2.16 (11) (anti-I-A^k), and JIID (12) as described (9, 13).

Cell Preparation. Thymocytes were fractionated with peanut agglutinin (PNA) lectin into cortical and medullary populations as described by Kruisbeek and Astaldi (14). Cortisone-resistant thymocytes were obtained 48 hr after *in vivo* i.p. administration of 1.5 mg of cortisone acetate. Spleen and thymus were fractionated on discontinuous bovine serum albumen gradients by the method of Steinman and Cohn (15) and were exposed to 1660 R of γ-irradiation. Antibody-plus-complement treatment of cell populations was performed as described (16).

Cell Culture. Cultures were prepared in a final volume of 200 μl or 1 ml of RPMI 1640 medium supplemented as described (13) but also containing 0.25 μM indomethacin. Thymocytes or thymocyte subpopulations were cultured with or without X-irradiated density-fractionated cell populations. The specific number of each cellular component is indicated in the respective experimental protocols. Cultures were incubated at 37°C for the indicated length of time; the supernatant was removed and/or incorporation of [^{3}H]thymidine into DNA was assessed as described (17).

Assay of Cytokines. IL-1 was assayed by the method of Mizel *et al.* (18). IL-2 was assayed as described (13). IL-2 was selectively removed from culture supernatant by the method of Smith (19).

RESULTS

Thymocyte Induction of IL-1. Supernatant derived from coculture of medullary thymocytes with irradiated accessory cells was examined for mitogenic activity in an IL-1 assay. A soluble mitogenic activity was measurable only upon interaction of the two cell populations (Fig. 1). No activity was detectable in cultures of thymocytes or accessory cells cultured separately (Figs. 1–3).

The IL-1 assay is sensitive to the two cytokines IL-1 and IL-2. To assess the respective contribution of these factors to the observed mitogenic activity, we also assayed the IL-2 content of the thymocyte culture supernatants. The IL-2 assay is insensitive to IL-1 and is more sensitive to IL-2 than is the IL-1 assay (unpublished data). Several observations show that IL-2 did not account for the activity detected in our cultures. IL-2 was not always detectable in active supernatants and, when present, was not found in sufficient amounts to be detectable in the IL-1 assay (Fig. 2 and data not shown). Further, when this low level of IL-2 was selectively removed from the supernatant, activity was still detectable in the IL-1 assay (Fig. 2). Therefore, IL-2 is neither necessary nor sufficient to account for the activity present in the thymocyte/accessory cell supernatants. By exclusion, we conclude that IL-1 is present in our culture supernatants.

Direct Proliferation of Thymocytes. Addition of IL-1 into thymocyte cultures results in proliferation that is maximal at

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MHC, major histocompatibility complex; IL-1 and -2, interleukins 1 and 2; PNA, peanut agglutinin; MAb, monoclonal antibody.

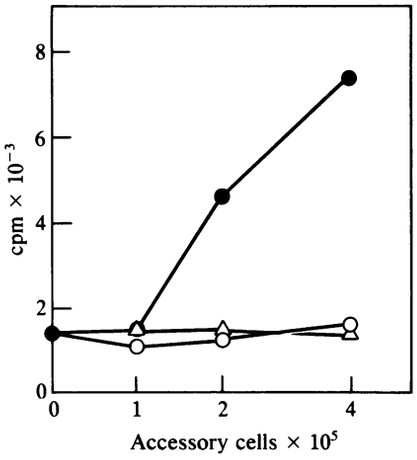

FIG. 1. Thymocyte induction of IL-1. An IL-1 assay was performed on a 1:4 dilution of supernatants from 200-μl cultures (incubated 120 hr) of 1 × 10⁶ C3H/Fej cortisone-resistant thymocytes with or without the indicated numbers of X-irradiated syngeneic low-density (●) or high-density (○) splenic cells or from low-density splenic cells cultured alone (△).

72 hr (9, 20). Because it appears that thymocytes are capable of inducing IL-1 in culture, we examined whether thymocyte proliferation ensued. Both IL-1 generation and thymocyte proliferation were assayed on the same cultures as a function of time. IL-1 was detectable after 96 hr of culture and subsequently was followed by thymocyte proliferation (Fig. 3).

Characteristics of the Interacting Cell Subsets. Both the generation of IL-1 and the direct thymocyte proliferation require an accessory cell. The requisite accessory cell for both responses is radioresistant and present only in the low-density ($\rho < 1.082$) cell fractions from spleen (Figs. 1 and 4). This cell fraction (2–5% of the initial cell population) is greatly enriched for macrophages and dendritic cells. The relevant accessory cell is also present in normal thymus (Fig. 5).

The nature of the thymocyte capable of proliferating upon interaction with accessory cells was characterized with respect to its maturity. Both cortical (immature) and medullary (mature) thymocytes proliferated upon coculture with accessory cells (Fig. 5). Medullary thymocytes proliferated to a much greater extent than did cortical cells, whose response was the same as that of unfractionated thymocytes. This greater response of the mature cells was not attributable to the lectin exposure during cell fractionation, as cells isolated

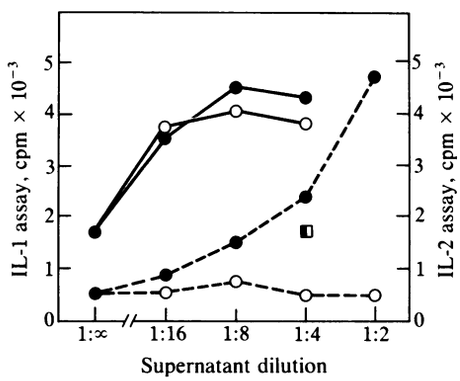

FIG. 2. IL-1 and IL-2 assays on thymocyte culture supernatants. ○, IL-2 absorbed; ●, unabsorbed. Supernatants were generated from 96-hr, 1-ml cultures of 5 × 10⁶ C3H/Hej PNA⁻ thymocytes with 5 × 10⁶ X-irradiated splenic low-density cells (●, ○) or from either cell population cultured alone (■, □). Supernatants were tested without further manipulation (●, ■) or after removal of IL-2 by bioabsorption (○, □) in an IL-1 (—, ■, □) or IL-2 (----) assay.

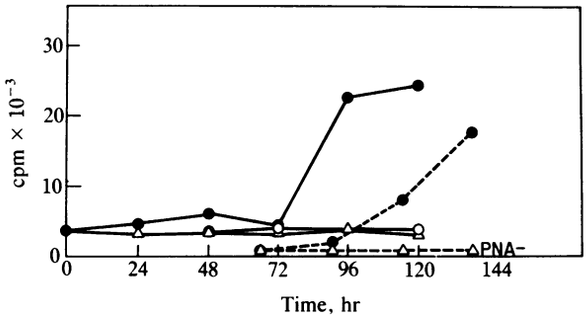

FIG. 3. Kinetics of IL-1 generation and thymocyte proliferation. PNA⁻ C3H/Fej thymocytes, (5 × 10⁵) and X-irradiated low-density splenocytes (4 × 10⁵) were cultured by themselves (△, ○, respectively) or together (●) in 200 μl. At the indicated times, 50 μl of supernatant was removed and tested for IL-1 activity (—), and the proliferation in the same culture wells was measured (----). The culture supernatant was assayed at a 1:4 final dilution.

on the basis of steroid resistance were similarly responsive (Fig. 1; unpublished data). The weaker response of cortical cells is not due to contamination of this cell fraction with medullary thymocytes, as the responding cell in such cultures bears an immature cell marker as assessed by treatment with the JIID MAb and complement (ref 12; data not shown). This pattern of activity exactly paralleled the responsiveness of these subpopulations to exogenous IL-1 (9).

The Role of MHC Gene Products in IL-1 Induction and Thymocyte Proliferation. We demonstrated that addition of IL-1 to thymocyte cultures results in the activation of thymocytes with specificity for self-Ia molecules (9). Because Ia molecules may be involved in the induction of IL-1, we postulated that the autoamplification of cells with specificity for autologous class II MHC molecules could occur. To test the involvement of Ia molecules in thymocyte proliferation to syngeneic accessory cells, we performed several experiments. First, the requisite accessory cell was eliminated by treatment with anti-Ia MAb and complement (data not shown). Second, anti-Ia MAb was added directly to thymo-

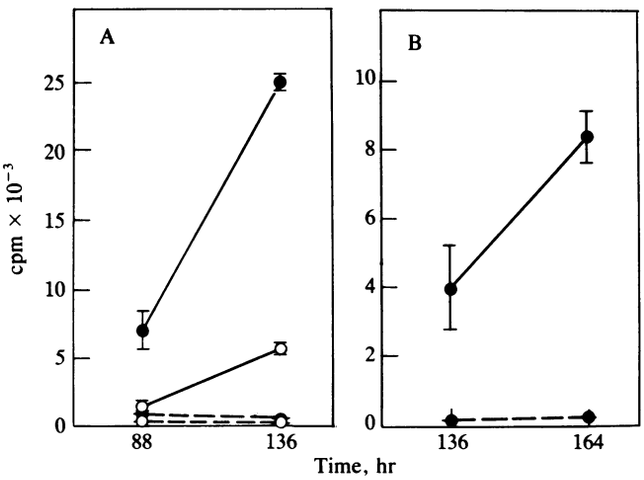

FIG. 4. Direct proliferation of thymocyte T-cell subsets to syngeneic accessory cells. (*A*) Microcultures (200 μl) were prepared with 1.5 × 10⁵ C3H/Hej unfractionated thymocytes (○) or 5 × 10⁵ PNA⁻ thymocytes (●) with (—) or without (----) 4 × 10⁵ X-irradiated splenic low-density accessory cells. (*B*) Microcultures with 1.5 × 10⁶ PNA⁺ C3H/Hej thymocytes with (—) or without (---) 4 × 10⁵ X-irradiated splenic low-density cells. Cultures were incubated for the indicated length of time, and incorporation of [³H]thymidine into DNA was assayed over the last 18 hr of culture. The results of cultures with high-density cells were identical to those without accessory cells; therefore, the data points have been omitted for clarity.

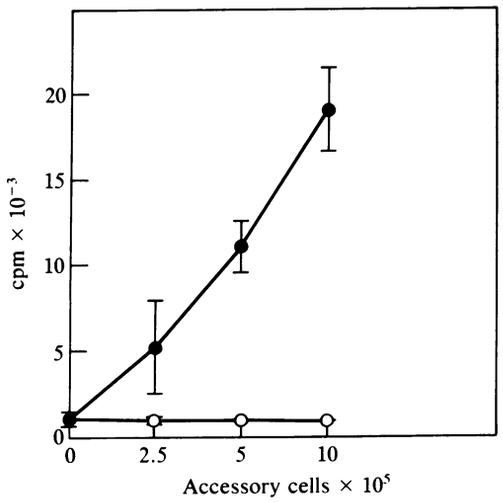

FIG. 5. Comparison of the accessory cell function of low-density (●) and high-density (○) thymic accessory cells in the direct activation (proliferation) of thymocytes. Microcultures were prepared with 5 × 10^5 PNA⁻ thymocytes with the indicated numbers of X-irradiated low- or high-density thymic accessory cells and incubated for 135 hr as described in Fig. 4.

cyte cultures, and activation of thymocytes was specifically blocked under these conditions (Fig. 6). Identical results were seen with cultures of cortical thymocytes (unpublished data). Third, the effect of anti-Ia MAb upon the thymocyte induction of IL-1 was tested. Inclusion of anti-Ia MAb in thymocyte-accessory cell cocultures specifically blocked the generation of IL-1 (Fig. 7).

DISCUSSION

The major findings of this report are: (*i*) proliferation of self-MHC class II antigen (Ia antigen)-specific thymocytes occurs upon interaction with accessory cells *in vitro*; (*ii*) self-Ia-specific thymocytes induce IL-1 in culture; (*iii*) after IL-1

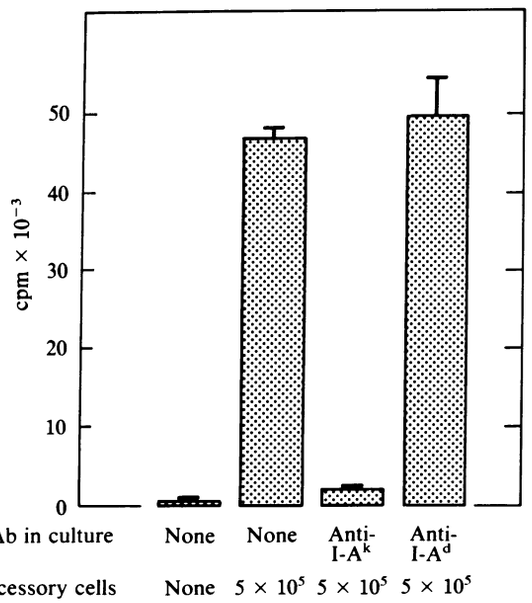

FIG. 6. The direct proliferative response (▨) of PNA⁻ thymocytes (*H-2ᵏ*) to syngeneic accessory cells is blocked by anti-I-A MAb. Microcultures were prepared with 10^6 C3H/Fej PNA⁻ thymocytes with or without 5 × 10^5 X-irradiated low-density splenic accessory cells in the presence or absence of the indicated MAbs (anti-I-Aᵏ = 10.2.16; anti-I-Aᵈ = MKD6) and incubated for 136 hr.

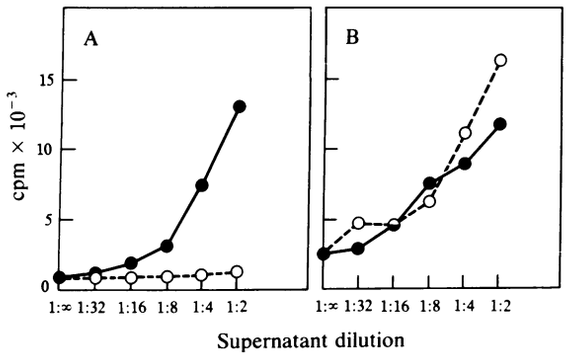

FIG. 7. IL-1 production is inhibited by anti-I-A MAb. Supernatants from 120-hr incubations were generated as described in Fig. 2 except with 2.5 × 10^6 of each cell population in the absence (●) or presence (○) of anti-I-Aᵈ (MKD6) (*B*) or anti-I-Aᵏ (10.2.16) (*A*) MAb. The IL-1 content of these supernatants were assayed with both BALB/c (*H-2ᵈ*) and C3H/Hej (*H-2ᵏ*) cells (*A* and *B*, respectively) so as to control for potential inhibition of the IL-1 assay by the anti-I-A MAbs.

induction, thymocyte proliferation ensues; and (*iv*) Ia molecules are criticaly involved in these processes.

The generation of IL-1 in this system requires a thymocyte–accessory cell interaction. Culture of either cell alone results in no detectable activity. Although IL-2 is measurable in some of the cultures, it is neither necessary nor sufficient to account for the activity measured in the IL-1 assay. We therefore refer to the activity detected as IL-1, although molecular identity with that factor has not been proven formally. IL-1 is a monokine and is presumably produced by the accessory cells in our cultures. Supporting this interpretation, we have observed similar levels of activity from parallel cultures of accessory cells with thymocytes or endotoxin, a nonspecific IL-1-inducing agent (unpublished data).

Upon induction, IL-1 appears to be rapidly produced, being first detected at 96 hr at almost maximal levels. Proliferation follows at ≈115 hr and becomes maximal at 136–164 hr. Of importance is the finding that the proliferation occurs in the absence of exogenous added cytokines. Although *in vitro* thymocyte autoproliferation has been reported, it has been observed to occur only without added exogenous factors in the neonate (21–24). It would appear that thymocytes are capable of inducing IL-1 and responding to it. Thymocyte proliferation to added exogenous IL-1 peaks at 72 hr, which is the approximate interval between the appearance of IL-1 and the maximal proliferation that is observed above. The response to exogenous added factor is identical to the direct proliferative response described here with respect to the nature of the accessory cell requirement, the responsiveness of the various thymic T-cell subsets, and specificity of the response. Therefore, it seems probable that these responses with and without factor represent the same phenomenon for which IL-1 generation is rate-limiting. Such a limitation has been suggested for other T-cell responses (17).

The thymocyte response to IL-1 is not a direct mitogenic effect of this factor. Our previous studies have shown that thymocytes recognize autologous class II MHC molecules on syngeneic accessory cells and, in the presence of IL-1, are driven to proliferate (9). MHC molecules also play a critical role in the induction of IL-1. Anti-Ia MAbs specifically block the generation of IL-1 in culture. Based on these findings, it is likely that the direct thymocyte proliferation is inhibited by such MAbs through blockage of the two required activation signals.

This MHC-specific spontaneous thymocyte proliferation is observed with both mature and immature thymocytes. Therefore, the phenomenon occurs at ontogeneic stages at

which the forces that shape the T-cell repertoire are thought to act. The medullary thymocytes respond more strongly than the cortical cells. The reason for this difference is not defined by our data. There are many possible reasons that could account for this observation, including precursor frequency, receptor affinity or avidity, and functional maturation. Because medullary thymocytes are derived from cortical cells, these differences could reflect a selective expansion.

We suggest that the thymocyte IL-1 response may be of relevance to thymic specification of the T-cell repertoire (9). It had previously been proposed that one of the major strategies of this process is the selective expansion of thymocytes on the basis of receptor specificity by an undefined mechanism (4–8). Perhaps the most conservative biological mechanism would be to utilize the same set of activation signals that subsequently would be used by the mature cell. The results of this report, along with our previous observations, suggest that the thymocyte and the mature post-thymic T cell have similar activation requirements (9). Further, the ostensible receptor specificity in the thymus is for self-MHC molecules. Because Ia molecules on accessory cells may serve as an induction signal for IL-1, a linkage of required activation signals could selectively amplify cells with self-specificity (16, 25, 26). The system could start with T cells of random specificity and, by activational constraints, generate a self-restricted repertoire. Our finding that anti-Ia MAbs block both IL-1 induction and thymocyte proliferation shows that Ia molecules are involved in the generation of both activation signals and is consistent with such a mechanism. The result of the thymocyte–accessory cell interaction appears to be an autoamplification of a selected specificity. It is notable that the *in vivo* generation of the T-cell repertoire is strongly correlated with the MHC genotype of the thymic accessory cells and is blocked by anti-Ia MAbs (27, 28).

Although this mechanism would fit the available data, it remains to be proven formally that the requisite activation signals are linked and that this autoreaction will generate cells that will require restricted recognition of nominal antigens. In this context, it is of interest that *in vivo*, self-restriction appears to be acquired prior to experience with nominal antigen, which is consistent with autorecognition being the selection force (4). Further autoreactive cells are found in the peripheral T-cell pool, and these cells also seem to be antigen-specific (29). Alternate interpretations of our data exist, including the possibility that we are solely measuring high-affinity MHC self-specific cells, reactive with autologous antigens, that are not yet eliminated. The model is also incomplete. It would require additional mechanisms to delete the high-affinity MHC-specific cells that are generated. Once these autoaggressive cells are eliminated, the remaining lower-affinity cells, upon maturing, may no longer be triggered by unmodified Ia but may require the corecognition of Ia with a nominal antigen. A second mechanism for the generation of class I MHC-restricted cells would be required. *In vivo* experiments have not correlated class I MHC specification with thymic accessory cells (30). However, an overall similar mechanism could operate for the class I MHC-restricted repertoire, but presumably involving other activation signals.

The superb technical skills of Colette F. Gramm and especially of Mary Carol Mellen are gratefully acknowledged. The help of Mrs. Mary Jane Tawa in the preparation of this manuscript is gratefully acknowledged. This work was supported by Grants AI-14732 and 1R01-AI-CA-20248-01 and by Training Grant 2T32-CA-09130-06 from the National Institutes of Health.

1. Miller, J. F. A. P. & Osoba, D. (1964) *Physiol. Rev.* **47,** 437–520.
2. Stutman, O. (1978) *Immunol. Rev.* **42,** 138–184.
3. Schwartz, R. H., Yano, A., Stimpfling, J. H. & Paul, W. E. (1979) *J. Exp. Med.* **149,** 40–57.
4. Zinkernagel, R. M. & Doherty, P. C. (1979) *Adv. Immunol.* **27,** 51–177.
5. Bevan, M. J. & Fink, P. J. (1978) *Immunol. Rev.* **42,** 3–19.
6. Jerne, N. K. (1971) *Eur. J. Immunol.* **1,** 1–9.
7. Von Boehmer, H., Hass, E. & Jerne, N. K. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2439–2442.
8. Langman, R. E. (1978) *Rev. Physiol. Biochem. Pharmacol.* **81,** 1–37.
9. Rock, K. L. & Benacerraf, B. (1984) *J. Immunol.*, in press.
10. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. (1981) *J. Exp. Med.* **153,** 1198–1214.
11. Oi, V., Jones, P., Goding, J., Herzenberg, L. A. & Herzenberg, L. A. (1978) *Curr. Top. Microbiol. Immunol.* **81,** 115–129.
12. Bruce, J., Symington, F. W., McKearn, T. J. & Sprent, J. (1981) *J. Immunol.* **127,** 2496–2501.
13. Rock, K. L. & Benacerraf, B. (1983) *J. Exp. Med.* **157,** 1618–1634.
14. Kruisbeek, A. M. & Astaldi, G. C. B. (1979) *J. Immunol.* **123,** 984–991.
15. Steinman, R. M. & Cohn, Z. A. (1974) *J. Exp. Med.* **139,** 380–397.
16. Rock, K. L. (1982) *J. Immunol.* **129,** 1360–1366.
17. Rock, K. L., Barnes, M. C., Germain, R. N. & Benacerraf, B. (1983) *J. Immunol.* **130,** 457–462.
18. Mizel, S. B., Oppenheim, J. J. & Rosenstreich, D. L. (1978) *J. Immunol.* **120,** 1497–1503.
19. Smith, K. A. (1980) *Immunol. Rev.* **51,** 337–357.
20. Gery, I., Gershon, R. K. & Waksman, B. H. (1972) *J. Exp. Med.* **136,** 128–142.
21. Von Boehmer, H. & Adams, P. B. (1973) *J. Immunol.* **110,** 376–383.
22. Howe, M. L. (1973) *J. Immunol.* **110,** 1090–1096.
23. Altman, A. & Katz, D. H. (1980) *J. Immunol.* **125,** 1536–1543.
24. Glimcher, L. H., Longo, D. L., Green, I. & Schwartz, R. H. (1981) *J. Exp. Med.* **154,** 1652–1670.
25. Rock, K. L. & Benacerraf, B. (1983) *Immunol. Rev.* **76,** 29–57.
26. Gilman, S. C., Rosenberg, J. S. & Feldman, J. D. (1983) *J. Immunol.* **130,** 1236–1240.
27. Longo, D. L. & Schwartz, R. H. (1980) *Nature (London)* **287,** 44–46.
28. Kruisbeek, A., Fultz, M. J., Sharrow, S. O., Singer, A. & Mond, J. J. (1983) *J. Exp. Med* **157,** 1932–1946.
29. Rock, K. L. & Benacerraf, B. (1983) *J. Exp. Med.* **157,** 359–364.
30. Zinkernagel, R. M. (1982) *J. Exp. Med.* **156,** 1842–1847.