Magnitude of response of histocompatibility-restricted T-cell clones is a function of the product of the concentrations of antigen and Ia molecules

(antigen presentation/immune response genes/major histocompatibility complex restriction)

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Contributed by William E. Paul, June 23, 1983

ABSTRACT The activation of specific T-cell clones by antigens is dependent upon the corecognition of restriction elements expressed on antigen-presenting cells (APC). For clones that respond by proliferation and lymphokine production, the restriction element is usually an Ia (I-region-associated) molecule. We show here that the magnitude of the proliferative response of such clones is a function of the product of antigen concentration and the number of Ia molecules expressed on APC. This conclusion was reached through the study of antigen concentration–response curves of T-cell clones specific for pigeon cytochrome *c*. These curves are characterized by a peak in thymidine incorporation, followed by a decrease in the magnitude of the response as antigen concentrations are increased. The decline in response at high concentrations was not the consequence of the emergence of suppressor cells in the APC population, as it was observed when cells of a cloned B-cell hybridoma line were used to present pigeon cytochrome *c* to these T-cell clones. The critical role of both antigen concentration and the number of Ia molecules on the APC in determining the magnitude of proliferation was demonstrated in several ways. (i) An inverse relationship was observed between the antigen concentration required for maximum proliferation and the number of APC present in culture. At high antigen concentrations, which caused responses less than maximum, reducing the number of APC actually increased the magnitude of the antigen-induced proliferation. (ii) Decreasing the number of relevant Ia molecules per APC (i.e., as in F₁ hybrids) resulted in the requirement for an increased antigen concentration for maximal response and in enhanced proliferation at high antigen concentrations. (iii) In the presence of anti-Ia antibody, higher concentrations of antigen were required for maximal response; at high antigen concentrations, proliferation was enhanced in the presence of a monoclonal anti-Ia antibody directed against the Ia restriction element.

Activation of antigen-specific regulatory T lymphocytes requires concomitant recognition of antigen and of I-region-associated Ia molecules expressed on antigen-presenting cells (APC). In examining the antigen-induced proliferation of T-cell populations specific for pigeon cytochrome *c*, we have previously presented evidence that the concentration of antigen and the number of available Ia molecules together determine the magnitude of the *in vitro* proliferative response (1). Specifically, higher concentrations of antigen were required to cause maximal degrees of proliferation by pigeon cytochrome *c*-specific, major histocompatibility complex (MHC)-restricted T-cell lines when APC populations expressing fewer of the relevant Ia molecules per cell were used. In this report we demonstrate, using cloned T-cell lines, that the magnitude of antigen-in-

duced proliferation is a function of the product of the antigen concentration and the number of Ia molecules expressed on APC.

We further observed that in these cytochrome *c*-specific T-cell clones, the magnitude of proliferation achieved a maximum and then declined as a function of the product of antigen concentration and APC number. Previous observations with T-cell populations had revealed that proliferative responses decreased at higher antigen concentrations (2). However, in those studies the possibility could not be eliminated that the diminished responses to high antigen concentrations were the consequence of suppressive interactions between distinct subpopulations of responding cells. In the experiments presented here, cloned T-cell lines and, in some experiments, cloned B lymphoma lines as APC were used. The results indicate that the observed diminution in response at high antigen concentration is an intrinsic property of the responding T cell.

METHODS AND MATERIALS

Animals. B10.A(5R)/SgSn [B10.A(5R)] and B10.A/SgSn (B10.A) mice were obtained from The Jackson Laboratory. B10.S(9R)/Sf and B10.PL/Sg mice were the progeny of breeding pairs provided by Jack Stimpfling (McLaughlin Research Institute, Great Falls, MT). F₁ hybrids were bred in our animal colony from these B10 congenic lines.

Antigens and Immunization. Cytochromes *c* were either purchased from Sigma or were the gift of C. Hannum and E. Margoliash (Northwestern University, Evanston, IL). They were purified by column chromatography on carboxymethylcellulose to remove contaminants such as polymeric and deaminated cytochrome *c* (3). The preparation of cyanogen bromide cleavage fragments of cytochrome *c* has been described (4). Mice were immunized as reported with 8 nmol of pigeon cytochrome *c* in the hind foot pads (2).

Preparation and Assay of Antigen-Specific Long-Term T-Cell Lines and Clones. Long-term T-cell lines were established from draining lymph nodes of immunized mice as described (2). T-cell clones were derived from long-term lines by limiting dilution (5). The proliferative responses of antigen-specific clones were assayed by culturing 10⁴ T cells per well in 96-well flat-bottom microtiter plates (Costar 3524, Cambridge, MA) with various doses of antigen or medium alone and various numbers of APC in a final 200-μl volume per well. After 1, 2, or 3 days, cultures were pulsed with 1 μCi (1 Ci = 37 GBq) per well of tritiated thymidine (6.7 Ci/mmol, New England Nuclear) and

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Abbreviations: APC, antigen-presenting cell(s); cyto *c* 81–104, pigeon cytochrome *c* fragment 81–104; THWM, tobacco hornworm moth.

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harvested 12–18 hr later. Incorporation of radioactivity was measured in triplicate cultures; and the data are expressed as the arithmetic mean $\pm$ SEM; Δ cpm represents the difference between the means of antigen-stimulated and nonstimulated cultures. Splenic APC were single-cell suspensions of spleen cells (from unimmunized mice) irradiated with 3,300 R. The B cell–B lymphoma hybridoma, TA3, is a fusion product of a lipopolysaccharide-activated (BALB/c $\times$ A) F_1 B cell with a drug-marked variant of the BALB/c B lymphoma M12.4.1 and was prepared by T. Hamano (Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases) by a previously described procedure (6). It expresses the I-region encoded I-A^{d/k}:I-E^{d/k} Ia molecules. The APC function of TA3 was assayed by adding various numbers (e.g., 10^3 , 10^4) of irradiated (10,000 R) TA3 per well to cultures of 10^4 responding T cells with or without antigen.

Monoclonal Antibody. The preparation and characterization of the monoclonal anti-Ia antibody Y-17 has been described (7). Its blocking activity was assessed by adding 1% (vol/vol) of the 50% saturated ammonium sulfate precipitate of Y-17 containing ascites to cell cultures. The Y-17 used in the studies was the generous gift of E. Lerner and C. Janeway, Jr. (Departments of Pathology and Cell Biology, Yale University School of Medicine, New Haven, CT).

RESULTS

Biphasic Proliferative Response of T-Cell Clones. The response of T cells from H-2 congenic B10.A (H-2^a) and B10.S(9R) (H-2^k) mice to pigeon cytochrome *c* requires the presence of APC that express E_B:E_A Ia molecules (2, 8). B10.A clone A3.G4 is an Lyt 1⁺2⁺ T-cell clone capable of proliferating when presented with pigeon cytochrome *c* in the context of either the E_B:E_A or E_B:E_k Ia molecule, expressed on B10.A or B10.S(9R) APC, respectively (5). In the presence of 5×10^5 B10.A APC, the antigen concentration–response curve of this clone is characterized by a peak response at 0.024 μ M and by a 75% decrease in the magnitude of proliferation relative to the peak response, at 2.4 μ M (Fig. 1). However, when 5×10^5 B10.S(9R) APC were used, the antigen concentration–response curve was shifted; higher concentrations (2.4 μ M) of antigen were required to stimulate peak responses. These data indicate that the diminished response of cloned T cells observed at high antigen concentrations in the presence of B10.A APC could not be due to a direct suppressive effect of high concentrations of antigen because the clone mounts its peak response to such concen-

FIG. 1. Antigen concentration-induced proliferative response of B10.A clone A3.G4 in the presence of B10.A versus B10.S(9R) splenic APC. T cells (10^4 cells per well) were cultured with various concentrations of cyto *c* 81–104 or medium alone in the presence of 5×10^5 irradiated (3,300 R) splenic APC. Proliferation was assessed in a 4-day-tritiated thymidine incorporation assay. Medium controls were: B10.A APC, 115 ± 35 ; B10.S(9R) APC, 95 ± 6 .

trations of antigen in the presence of B10.S(9R) APC. These results also strongly suggest that Ia molecules expressed on APC play a critical role in determining the magnitude of the T-cell proliferative response. The shape of the antigen concentration–response curves did not vary when thymidine incorporation was measured at successive time intervals during the first 3 days after the initiation of cell culture (data not shown). Thus, the diminished proliferation observed at high antigen concentrations was not simply the consequence of differences in the kinetics of the response at distinct antigen concentrations.

T-Cell Proliferation Is a Function of the Product of the Antigen Concentration and the Number of APC. In order to explore the means through which the number of Ia molecules influences the magnitude of antigen-induced T-cell proliferation, antigen concentration–response curves of A3.G4.F3, a subclone of A3.G4, to pigeon cytochrome *c* fragment 81–104 (cyto *c* 81–104) were measured in the presence of various numbers of irradiated B10.A splenic APC (Fig. 2A). The antigen concentration at which the peak response was obtained was inversely related to the number of APC present. In the presence of 10^6 APC, the maximal response was observed at an antigen concentration of 0.024 μ M, whereas maximal proliferation in the presence of 10^5 or 5×10^4 APC was observed at 2.4 μ M. Furthermore, the shift in concentration required for maximal response was paralleled by a shift in the concentration required for inhibition. Indeed, in the presence of 24 μ M cyto *c* 81–104, the proliferative response was actually enhanced as the number of APC was decreased from 10^6 to 10^5 . There was also a tendency for the maximum response to decrease in magnitude below a certain number of APC. The degree of this diminution varied from one experiment to another.

The shift of the antigen concentration–response curve with changes in the number of APC suggested that the response depended on the product of these two elements. To examine this, we plotted the magnitude of proliferation as a function of the product of the cyto *c* 81–104 concentration and the number of APC (Fig. 2B). Although there is some scatter near the peak response, individual points on a plot of the product of (concentration of cyto *c* 81–104) $\times$ (number of APC) against the normalized response appear to fall on a smooth curve. The maximal response was obtained with a product of 5×10^4 μ M·cells.

Concentration–response curves were repeated with a cloned population of APC in the form of a B lymphoma–B cell hybridoma (TA3) (Fig. 2C). These cells provide a homogeneous population of APC. In addition, because they represent a cloned line, they are devoid of any T cells or T-cell precursors, providing a more stringent test of a potential role for suppressor T cells in the inhibition seen at high concentrations. Antigen concentration–response curves to cyto *c* 81–104 in the presence of 10^4 and 10^3 TA3 APC per microtiter well are shown in Fig. 2C. In the presence of 10^4 TA3 APC, peak proliferation was observed at 0.0024 μ M antigen, and there was an 80–85% diminution, relative to the maximum, at 24 μ M antigen. Lowering the number of TA3 cells to 10^3 per well shifted the concentration–response curve of clone A3.G4; higher antigen concentrations were required to obtain maximal response. In addition, the shifted concentration–response relationship led to a much greater response to 24 μ M cyto *c* 81–104 in the presence of 10^3 TA3 cells than in the presence of 10^4 APC (Fig. 2C).

The T-Cell Response Is Also a Function of the Number of Ia Molecules Expressed per APC. It was of interest to determine whether antigen concentration–response curves would similarly shift when the number of APC remained constant but the amount per APC of the Ia molecule required for cyto *c* 81–104 presentation was varied. The data in Fig. 3 depict antigen concentration–response curves of A3.G4 in the presence of

FIG. 2. (A) T cells (10^4) were cultured in the presence of varying numbers of B10.A APC, with graded numbers of nonresponder irradiated splenic filler cells from B10 mice added to achieve a final number of 10^6 APC per well in all wells. Medium controls in cpm $\pm$ SEM were: 10^6 APC, 131 ± 11 ; 5×10^5 APC, $2,606 \pm 1,279$; 2.5×10^5 APC, 865 ± 155 ; 10^4 APC, 328 ± 143 ; 5×10^4 APC, 241 ± 9 . (B) Data from A, represented as proliferation versus the product of antigen concentration and the number of APC. Since different maximum responses were obtained using different numbers of APC, the proliferative response corresponding to each product is normalized and expressed as the fraction of the maximum response obtained for that number of APC. The number of APC represented by each of the points in the figure is as follows: Δ , 10^6 APC; $\circ$, 5×10^5 APC; $\square$, 2.5×10^5 APC; $\diamond$, 10^4 APC. (C) Antigen concentration-induced proliferative response of B10.A clone A3.G4 in the presence of 10^4 versus 10^3 irradiated (10,000 R) TA3 B lymphoma-B cell hybridoma cells used as APC. Medium controls were: 10^4 TA3, 736 ± 27 ; 10^3 TA3, 374 ± 101 .

B10.A APC and APC from [B10.A(4R) $\times$ B10.A(5R)]F₁ (4R $\times$ 5R)F₁ ($E^k_\beta:E^k_\alpha$) and [B10.A(4R) $\times$ B10.PL]F₁ (4R $\times$ PL)F₁ ($E^k_\beta:E^u_\alpha$) hybrid mice (Fig. 3A). These APC express different amounts of an $E^k_\beta:E^k_\alpha$ molecule required for pigeon cytochrome c recognition by B10.A T cells (9). It has been shown by quantitative immunofluorescence that cells from responder F₁ hybrids prepared by mating nonresponder B10.A(4R) mice, which possess only the E^k_β allele required for pigeon cytochrome c recognition, and other nonresponder strains, which possess any one of a number of structurally similar E_α alleles (e.g., E^k_α , E^d_α , E^r_α , E^p_α), generally express about 1/2 the number of functionally competent $E^k_\beta:E^k_\alpha$ Ia molecules as do homozygous responder strains (9). Although the E_α chain is serologically similar to the other E_α chains, a quantitative defect exists in the expression of the $E^k_\beta:E^u_\alpha$ Ia molecule in the (4R $\times$ PL)F₁ hybrid.

FIG. 3. (A) Antigen concentration-response curve of clone A3.G4 in the presence of equal numbers (5×10^5) of B10.A APC ($\circ$), (4R $\times$ 5R)F₁ APC ($\square$), or (4R $\times$ PL)F₁ APC ($\triangle$). Proliferation assay was performed as in the legend to Fig. 1. Medium controls in cpm $\pm$ SEM were: B10.A APC, 511 ± 317 ; (4R $\times$ 5R)F₁ APC, 157 ± 10 ; (4R $\times$ PL)F₁ APC, 629 ± 25 . (B) The data in A presented as proliferation versus the product of antigen concentration and APC number. An effective APC number is calculated by multiplying the actual B10.A APC number (5×10^5) by the relative number of $E^k_\beta:E^k_\alpha$ Ia molecules expressed on each APC-type as determined by quantitative immunofluorescence (9). (APC equivalents). The ratios of $E^k_\beta:E^k_\alpha$ Ia molecule expressed per APC are approximately as follows: B10.A/(4R $\times$ 5R)F₁/(4R $\times$ PL)F₁, 1:0.5:0.125.

These APC express only 1/8 as many $E^k_\beta:E^k_\alpha$ Ia molecules as do B10.A mice (9). APC from B10.A mice and from both F₁ hybrids were competent to present cyto c 81-104 to A3.G4 (Fig. 3A). However, the quantitative differences in Ia molecule expression per well resulted in a requirement for higher antigen concentrations to obtain maximum responses when (4R $\times$ PL)F₁ APC were used, in comparison to the antigen concentration required for maximum response in the presence of the same number of (4R $\times$ 5R)F₁ APC or of B10.A APC. Furthermore, the relative degree of the inhibition of proliferation at the highest antigen concentration (24 μM) was inversely related to the number of $E^k_\beta:E^k_\alpha$ molecules per cell (Fig. 3A). These data may also be represented by plotting the normalized magnitude of proliferation as a function of the product: (antigen concentration) $\times$ (number of B10.A APC). APC number of (4R $\times$ 5R)F₁ and (4R $\times$ PL)F₁ were reduced by factors of 2 and 8, respectively, to account for the diminished number of $E^k_\beta:E^k_\alpha$ Ia molecules expressed on the F₁ hybrid APC (Fig. 3B; Fig. 3 legend). Again, the points appear to follow a smooth curve.

Another way of controlling the effective number of Ia molecules available for antigen presentation was by introducing anti-Ia antibodies into the culture. The data in Fig. 4 represent antigen concentration-response curves of the pigeon cytochrome c-specific B10.S(9R) clone 9R.F4:F2 in the presence or absence

of the monoclonal anti-Ia antibody Y-17, which is specific for a combinatorial determinant on the $E_\beta:E_\alpha$ Ia molecules expressed on both B10.S(9R) and B10.A APC. The antigen-stimulated proliferative response of 9R.F4.F2 in the presence of syngeneic B10.S(9R) APC was completely inhibited in the presence of Y-17 at 0.24 μ M cyto *c* 81-104 (Fig. 4A). In contrast, the proliferative response of this clone at 24 μ M cyto *c* 81-104 actually was enhanced in the presence of anti-Ia antibody (Fig. 4A). Thus, the anti-Ia antibody effectively shifted the antigen concentration response curve to higher antigen concentrations. This result presumably reflects the decreased number of Ia molecules available to the T-cell receptors in the presence of the anti-Ia antibody. Clone 9R.F4.F2 also has been shown to be alloreactive against the homologous $E_\beta:E_\alpha$ Ia molecule expressed on B10.A APC and to manifest an antigen-specific proliferative response above the alloreactive background as a result of the corecognition of cyto *c* 81-104 and the $E_\beta:E_\alpha$ restriction element (Fig. 4B). This antigen-specific response showed a biphasic antigen concentration-response curve with a diminished response at high antigen concentrations (Fig. 4B). Whereas both the alloreactive and the antigen-specific components of this response were completely inhibited by Y-17 when concentrations of cyto *c* 81-104 of 0-0.24 μ M were used, the proliferation at 24 μ M cytochrome *c* was significantly enhanced in the presence of the anti-Ia antibody (Fig. 4B).

Thus, the inhibition of T-cell proliferation at high antigen concentration may be reversed either by decreasing the number of APC (Fig. 2) or by decreasing the number of Ia molecules per APC (Figs. 3 and 4).

High Antigen Dose Inhibition of Proliferation by a Cross-reactive Species Variant Cytochrome *c*. Long-term T-cell lines and clones specific for pigeon cytochrome *c* in both B10.A and B10.S(9R) mice have been shown (10) to express a maximum *in vitro* proliferative response to lower concentrations of tobacco hornworm moth (THWM) cytochrome *c* than are required for a maximum response to pigeon cytochrome *c*. THWM cytochrome *c* possesses in common with the pigeon cytochrome *c* immunogen both a glutamine at amino acid residue 100 and a carboxyl-terminal lysine, which have been shown to contribute to the dominant antigenic determinant for T-cell recognition in these two strains (2). In contrast to pigeon cytochrome *c*, THWM cytochrome *c* has a deletion of alanine-103 and, therefore, bears

FIG. 4. Anti-Ia antibody shifts antigen concentration-response curve to higher concentrations of antigen. B10.S(9R) clone 9R.F4.F2 T cells were added (10^4 cells per well) with various concentrations of cyto *c* 81-104 and 5×10^5 irradiated (3,300 R) splenic APC in the presence or absence of 1% (vol/vol) Y-17 antibody. Δ cpm was determined in the 4-day tritiated-thymidine incorporation assay. Medium controls in cpm $\pm$ SEM were: B10.S(9R) APC and no Y-17, $1,047 \pm 351$; B10.S(9R) APC and 1% Y-17, 213 ± 33 ; B10.A APC and no Y-17, $34,034 \pm 1,763$; and B10.A APC and 1% Y-17, $1,072 \pm 209$.

FIG. 5. Inhibition of proliferation of pigeon cytochrome *c*-specific B10.S(9R) clone 9R.F4.F2 in the presence of THWM cytochrome *c*. T cells were cultured at 10^4 cells per well in the presence of 5×10^5 irradiated (3,300 R) syngeneic splenic APC and various concentrations of pigeon cytochrome *c*, THWM cytochrome *c*, or medium clone. Proliferation was assessed in a 4-day-tritiated thymidine incorporation assay. Medium control was $5,845 \pm 2,280$ cpm.

its carboxyl-terminal lysine at residue 103 rather than at residue 104. This enhanced proliferation of pigeon cytochrome *c*-primed T cells to the cytochrome *c* from THWM has been termed a heteroclitic response and has been characterized principally by a shift to lower antigen concentrations of the antigen concentration-response curve.

The concentration-response curves of clone 9R.F4.F2 to pigeon and THWM cytochromes *c* are depicted in Fig. 5. An enhanced proliferation to THWM as compared to pigeon cytochrome *c* was observed at lower antigen concentrations (0.0024 and 0.024 μ M). Conversely, the degree of inhibition of the response by THWM cytochrome *c* at 24 μ M antigen was significantly more pronounced. Thus, the inhibition of the T-cell proliferative response is not simply dependent upon the absolute concentrations of antigen and Ia molecules but, in addition, appears to be a function of the apparent affinity of the interaction(s) between the antigen and the Ia molecule with each other and with the combining site(s) of the T-cell receptor.

DISCUSSION

The series of experiments described in this report indicate that activation of I-region-restricted antigen-specific T-cell clones, as measured quantitatively by antigen-induced proliferation, is dependent on both the number of Ia molecules and the concentration of antigen and is consistent with the response being proportional to the product of the concentrations of antigen and Ia molecules. DNA synthesis in these clones can be inhibited by an excess of either antigen or Ia molecules. The magnitude of proliferation plotted as a function of the product of antigen concentration and the number of APC yields a biphasic concentration-response relationship with a single optimal value for maximum proliferation. The development of a mathematical model to account for these observations is currently in progress (D. Alling, unpublished data).

At least two mechanisms may be envisioned to account for the observed biphasic concentration-response relationships. One model would propose that T-cell activation occurs as a function of the number of cell surface receptors bound by ligand, such that maximal proliferation would correspond with a given number of receptor-ligand interactions per cell. Binding of additional receptors could result in inhibition of DNA synthesis by a separate mechanism, which is triggered only after larger numbers of receptors have been engaged. A possible precedent for

such a model derives from studies on the induction of histamine release from human basophils by anti-IgE antibodies (11). Although the release of histamine was shown to require bridging (crosslinking) of surface IgE molecules, maximal histamine release occurred in the presence of concentrations of anti-IgE antibodies at which little or no redistribution (patching and capping) of surface IgE was observed (11). Higher concentrations of anti-IgE which redistributed surface IgE most effectively in fact caused marked inhibition of histamine release. It was concluded that only small numbers of crosslinked cell surface IgE complexes effectively activated basophils to release histamine. In an analogous fashion, I-region-restricted T-cell activation may involve recognition of antigen-Ia molecule complexes on the surface of the APC (12, 13). Increasing either the antigen or the Ia molecule concentration would result in the formation of a greater number of such complexes, effectively increasing the ligand concentration and, thus, explaining the observed dependence of the magnitude of T-cell activation on the product of the concentration of antigen and the number of Ia molecules. It previously has been shown that for T-cell clones, such as A3.G4 (Figs. 1–3), which respond to antigen in the context of two or more distinct Ia molecules, the fine specificity of antigen-induced activation may vary markedly with the haplotype of the APC (5, 14). Such results also suggest that antigen and the Ia molecule must interact at least during the process of T-cell activation. Maximal T-cell DNA synthesis would then result when a given number of cell surface receptors are bound by antigen-Ia molecule complexes on the APC surface; relative inhibition of DNA synthesis would occur as additional T-cell receptors are engaged by the macrophage-bound complex ligand. However, the data presented here are also consistent with models in which antigen and the Ia molecule provide separate signals to the T-cell receptors, which are summed through the interaction of intracellular mediators.

An alternative explanation for the diminished proliferation of T-cell clones activated by high concentrations of antigen and/or excess Ia molecules derives from the observation that such antigen-activated proliferative T-cell clones produce a variety of lymphokines (15, 16). For example, it has been shown that several of the pigeon cytochrome *c*-specific T-cell clones produce both interleukin 2 (IL-2) and immune interferon upon antigen-induced stimulation and, in particular, that the antigen concentration-response curve for immune interferon production does not manifest the same high dose inhibition as does the proliferative response (16). In fact, higher concentrations of antigen and Ia molecules might increase the signal to the T-cell receptor. The consequent enhanced production of lymphokines with potential antiproliferative effects, such as immune interferon (17), could in turn account for the observed decreased magnitude of proliferation. Consistent with this explanation is the observation that the addition of exogenous interleukin 2 within 24–48 hr after initiation of the cell cultures was capable of reversing the antiproliferative effects of high antigen concentrations (unpublished observations).

In addition to the concentrations of antigen and Ia molecules, the dose-response relationships were influenced also by the amino acid sequence variants of these peptides recognized by the responding T cells. For example, the species variant cytochrome *c* from THWM, which induced enhanced proliferation of the pigeon cytochrome *c*-specific clones at low antigen concentrations, conversely caused more profound inhibition of proliferation at higher antigen concentrations (Fig. 5). Because the shift in the concentration response curve when THWM cytochrome *c* was used most likely reflects a higher affinity of the interaction between the antigen and the Ia molecule in the presence of the T-cell receptor, it would appear that the greater

inhibition of the response induced by higher concentrations of this species variant peptide is also a consequence of the higher affinity. This observation supports the conclusion that the induction of DNA synthesis and its relative inhibition at higher concentrations of antigen and/or Ia molecules are both consequences of a similar underlying recognition event.

The results presented here have shown that in Ia molecule-restricted antigen recognition by cloned T-cell lines, both the antigen concentration and the number of Ia molecules contribute in a quantitative fashion to T-cell activation. The magnitude of proliferation can be predicted as a function of the product of antigen concentration and the number of Ia molecules, implying that a physical product of antigen and Ia molecule is the critical driving force in T-cell activation. The demonstration that an excess of Ia molecules times antigen inhibits proliferation of antigen-specific T-lymphocyte clones implies a possible mechanism for the induction of T-cell tolerance. Experiments are currently in progress to determine whether prolonged exposure of such T-cell clones to high concentrations of antigens and Ia molecules renders them refractory to subsequent stimulation with either optimal concentrations of antigen plus APC or interleukin 2. The recently described (18) antigen-induced inhibition of human influenza virus-specific T-cell clones may represent an example of such a mechanism for tolerance induction.

The authors thank Dr. Charles DeLisi, Dr. David Alling, Dr. Stephen M. Hedrick, Dr. Jonathan Ashwell, and Dr. Dan L. Longo for productive discussions during the course of these studies, and Ms. Ann Lindgren for assistance in performing several experiments.

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