LIMITING DILUTION COMPARISON OF THE REPERTOIRES OF HIGH AND LOW RESPONDER MHC-RESTRICTED T CELLS

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Immune response (*Ir*) genes were originally discovered for responses to relatively simple polymers of a few amino acids (1), the simplest being poly-L-lysine, for which all-or-none responsiveness was seen in different inbred strains of guinea pigs (2). The same phenomenon of *Ir* gene control was then found to apply to more complex antigens such as natural proteins (3–8), but in these cases, high or low responsiveness rather than all-or-none was the rule. In earlier studies, we found evidence to support the hypothesis that the reason for high or low responsiveness rather than nonresponsiveness was that the responses to different epitopes on the same protein were under different *Ir* gene control (9–12). Thus, it was unlikely to find a strain of animals that lacked the response to all epitopes on a complex protein. Low responders merely responded to fewer epitopes.

For these reasons, recent studies on the mechanism of action of *Ir* genes have largely focused on responses to individual epitopes, most recently as represented by small synthetic peptides (8, 13–16). Two main types of mechanisms have been proposed and evidence has been presented to support each: holes in the T cell repertoire (17, 18) and preferential binding of different peptides to different MHC molecules (19–24). Probably both mechanisms can account for some of the *Ir* gene phenomena, and suppressive mechanisms may account for others (25).

Although some controversy remains about the control of responses to individual epitopes, enough is now known that it is time to return to the question of high vs. low responsiveness to whole proteins, which, after all, are what is encountered in nature during response to infection. This second look at whole proteins is also warranted by the fact that certain epitopes appear to be immunodominant (6, 8, 26–31). Therefore, we hypothesized that perhaps epitopes are not all created equal but, rather, the response to some so predominates that these alone will influence high or low responsiveness to the whole protein. Thus, it would not be simply the relative number of epitopes to which an individual could respond that would determine the magnitude of response to the protein, but rather the choice of the epitopes seen would play a role as great as or greater than that of the total number of epitopes. To study this question, we have undertaken a large project to compare the fine specificity T cell repertoire of a

high responder and a low responder to sperm whale myoglobin (SWMb).[1] For this purpose, we have combined classic limiting dilution analysis with the production of long-term limiting dilution T cell lines and the testing of fine specificity with synthetic peptides and cyanogen bromide cleavage fragments. The results indicate that high responsiveness depends primarily on the response to a major immunodominant epitope. The response to other epitopes never reaches the level of the response to this one and so does not compensate in strains that lack responsiveness to the immunodominant site. Therefore, although low responders respond to a number of epitopes, they remain low responders. In addition to the implications for *Ir* gene control of the response to natural proteins, these results support the notion that immunodominant epitopes are distinct from other epitopes in some important way.

Materials and Methods

Mice. C57BL/10 (B10), B10.D2, B10.BR, and (B10.BR × B10.D2)F_1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B10.A(4R), B10.GD, and B10.S were bred at our breeding facility from breeding pairs kindly provided by Dr. D. H. Sachs (National Institutes of Health) and Dr. D. Shreffler (Washington University, St. Louis, MO).

Antigens. SWMb, cyanogen bromide cleavage fragment 1–55, and synthetic peptides 102–118 and 132–146 of SWMb were prepared as previously described (4, 11, 32, 33).

Long-term Limiting Dilution Analysis. (B10.BR × B10.D2)F_1 mice were immunized with 100 μg of SWMb in CFA (1:1) subcutaneously in the tail. 8 d later, draining lymph node cells were collected, and T cells were enriched by passage through a nylon-wool column. They were stimulated in 24-well plates with 1.5 μM SWMb and rested one cycle on 3,300-rad irradiated F_1 spleen cells by the method of Kimoto and Fathman (34), except that only 10^6 irradiated spleen cells were used per well for the rest. The T cells were then plated in serial dilutions of 60 wells each at 800, 200, 40, 13, and 4 cells per well in 96-well microtiter plates with 5×10^5 irradiated spleen cells in the presence of SWMb and IL-2 (4 U/ml). Since responses of single precursor cells in the limiting dilution wells could not be detected, they were repeatedly stimulated and expanded, transferred to 24-well plates, and maintained as limiting dilution cell lines, each representing, within limits, the repertoire of precursors plated at the original limiting dilution cell numbers. Addition of IL-2 was stopped after a second stimulation in 24-well plates. Each line was tested for proliferation to SWMb, fragment 1–55, and peptides 102–118 and 132–146, and for recognition of these on presenting cells of congenic B10.D2 (A^d, E^d), B10.GD (A^d only), B10.BR (A^k, E^k), and B10.A(4R) (A^k only) strains. The results were calculated by a Poisson analysis using a least squares fit algorithm.

T Cell Proliferation Assay. The proliferative response of T cells was assayed as described previously (27) with some modifications. To increase the chances to detect the response of minor clones in each cell line, up to 1.5×10^5 cells were cultured with 2.5×10^5 irradiated spleen cells and antigens in microtiter wells. 3 d later, 1 μCi of [^{3}H]thymidine per 20 μl was added per well and harvested 18 h later onto glass fiber filters. The radioactivity uptake was measured by liquid scintillation counting.

Medium. Complete medium consisting of the following components was used in all cell cultures: 10% heat-inactivated FCS, 5×10^{-5} M 2-ME, 2 mM fresh L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco Laboratories, Grand Island, NY), 5% Hepes in a 1:1 mixture of RPMI 1640, and Eagle-Hank's amino acid medium made for 5% CO_2 conditions (35). All cultures were carried out in a humidified CO_2 incubator set at 5% CO_2, 37°C.

[1] *Abbreviation used in this paper:* SWMb, sperm whale myoglobin.

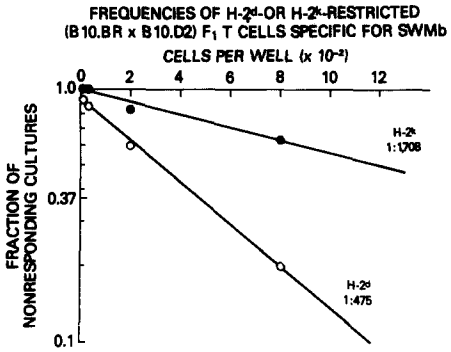

FIGURE 1. Frequencies of H-2^d– or H-2^k–restricted (B10.BR × B10.D2)F_1 T cells specific for SWMb. Long-term limiting dilution cell lines restricted to H-2^d or H-2^k, raised as described in Materials and Methods, were tested for proliferative response to 2 μM SWMb on each haplotype of APCs. The fraction of positive responding cell lines among 60 replicates of the original limiting dilution doses were subtracted from 1.0 and plotted as a function of dilution dose (800, 200, 40, and 13 per well). Lines were drawn by least square linearization, and frequencies were calculated from the point at which the fraction of nonresponding cultures was 0.37, according to Poisson statistics.

Results

SWMb-specific, Long-term Limiting Dilution Lymph Node Cell Lines. In vivo–primed (B10.BR [low responder] × B10.D2 [high responder])F_1 lymph node cells were stimulated and rested one round in vitro with F_1 APC to reduce autoreactive and nonspecific T cells and acclimate the cells to tissue culture. Serial dilution inocula (800, 200, 40, 13, and 4 cells per well) were then cultured with parental high or low responder APCs in the presence of IL-2 and antigen. These were repeatedly stimulated, expanded to levels at which they could be tested for responses to different peptides, and maintained as limiting dilution cell lines. It is possible that some precursors in any given well were lost during the expansion stage. However, since it was impossible to detect the responses of single cells, especially in multiple tests, we took each cell line as a statistically reasonable (over the whole study), if individually imperfect, representation of the precursors that went into that well.

Each cell line was initially tested for reactivity to SWMb. The results were calculated by Poisson analysis based on the number of cells per well in the original limiting dilution (Fig. 1). The frequencies of SWMb-specific T cells restricted to H-2^d or H-2^k at the time of the limiting dilution were 1:475 and 1:1,708, respectively. Thus, there was a 3.6-fold difference in the number of T cells between the high and the low responder MHC restrictions.

Comparison of Efficiency of (B10.BR × B10.D2)F_1 APC to Stimulate H-2^d– or H-2^k–restricted Cell Lines. Since limiting dilution was carried out after one round of stimulation and rest in vitro to adapt the cells to culture, and since the mice were immunized in vivo in the endogenous F_1 environment, the difference in the frequencies of H-2^d– or H-2^k–restricted T cells could be due to a different efficiency of expansion between H-2^d– and H-2^k–restricted T cells stimulated with F_1 APC and antigen before the limiting dilution. To test this possibility, we titrated the F_1 spleen cells for their efficiency in stimulating randomly selected H-2^d– and H-2^k–restricted cell lines from the limiting dilution. If cells of one of the restriction types show a much steeper response curve on titrating F_1 APC, an uneven expansion of the T cells probably occurred between the different restriction specificities in vivo and during one round of stimulation and rest in vitro before the limiting dilution. Although there were cell line–to–cell line differences, the result did not seem to show significant differences between the two restriction types (Fig. 2). Of course, we could study only those cell lines that

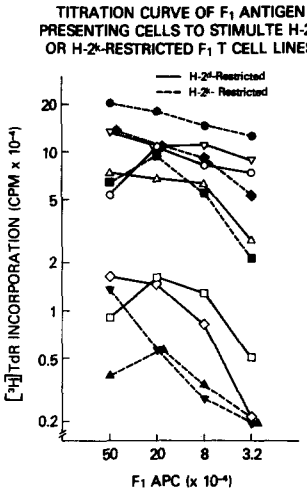

FIGURE 2. Titration of F_1 APC to stimulate H-2^d– or H-2^k–restricted F_1 T cell lines. Five each of randomly selected H-2^d– or H-2^k–restricted cell lines were cultured in microtiter wells (2 × 10^4 cells per well) with 2 μM SWMb and 5 × 10^5, 2 × 10^5, 8 × 10^4, or 3.2 × 10^4 of irradiated F_1 spleen cells per well. [^{3}H]Thymidine was added 3 d later, and its uptake was measured as described in Materials and Methods. Background levels were as follows: 28,000–92,000 cpm (○); 180–990 cpm (△); 190–420 cpm (□); 330–560 cpm (▽); 175–400 cpm (◇); 2,900–12,300 cpm (●); 280–570 cpm (▲); 145–410 cpm (▼); 640–41,490 cpm (◆); 26,900–103,600 cpm (■; which were completely autoreactive).

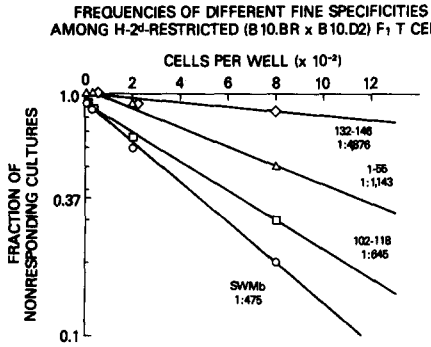

FIGURE 3. Frequencies of different fine specificities among H-2^d–restricted (B10.BR × B10.D2)F_1 T cells. The H-2^d–restricted cell lines were tested for proliferative response to 2 μM SWMb (○), 1 μM fragment 1–55 (△), 1 μM peptide 102–118 (□), and 1 μM peptide 132–146 (◇). For details, see legend for Fig. 1 and Materials and Methods.

grew. Thus, of necessity, there is an inherent selection for cells that grow under our culture conditions. With that qualification, if these are representative, we could not explain the frequency differences in the restriction types on the basis of greater sensitivity of one population to stimulation on F_1-presenting cells.

Fine Specificities of SWMb-specific H-2^d– or H-2^k–restricted F_1 T Cells. SWMb-specific B10.D2 clones and their fine specificities have been well studied (27, 28, 32, 33). Namely, dominant clones were specific for the epitope around glutamic acid at position 109 (Glu 109) and restricted to I-A^d; I-E^d–restricted T cells were far less frequent, and the majority of those clones is specific for lysine at position 140 (Lys 140). Some other clones respond to the cyanogen bromide cleavage fragment 1–55 on I-A^d, but finer mapping of their specificity is unknown.

Considering these facts, we tested the same limiting dilution T cell lines for the frequency of wells responding to different peptide fragments with different MHC restrictions. Fig. 3 shows the results of the fine specificities in H-2^d–restricted cell lines. The majority of SWMb-specific H-2^d–restricted T cells responded to the synthetic peptide of residues 102–118 (which includes Glu 109 and stimulates established Glu 109-specific T cell clones). The frequency was 1:645 compared with a total frequency in the experiment of 1:475 (i.e., 74%).

The T cells specific for peptide 132–146 were far less frequent (1:4,876) and

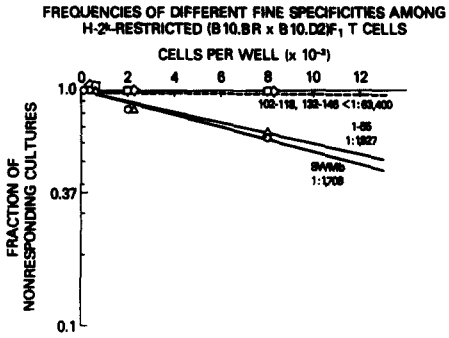

FIGURE 4. Frequencies of different fine specificities among H-2^k–restricted (B10.BR × B10.D2)F_1 T cells. The H-2^k–restricted limiting dilution cell lines were tested for SWMb (○), 1 μM fragment 1–55 (△), 1 μM peptide 102–118 (□), and 1 μM peptide 132–146 (◇). For details, see legends for Figs. 1 and 3.

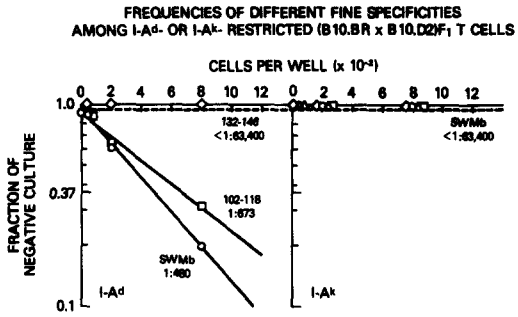

FIGURE 5. Frequencies of different fine specificities among I-A^d– or I-A^k–restricted (B10.BR × B10.D2)F_1 T cells. The H-2^d– or H-2^k–restricted T cell lines were tested as for response to SWMb or the peptides on B10.GD (A^dE^b) or B10.A(4R) (A^kE^b) spleen cells as APCs, respectively. Also 1 μg/ml Con A was tested to stimulate T cells on B10.A(4R) spleen cells, which gave thousands to 200,000 cpm of [^{3}H]thymidine incorporation. For details, see legend for Fig. 1 and Materials and Methods.

represented <10% of the SWMb-specific T cells, consistent with the magnitude of the bulk lymph node T cell response to 132–153 vs. whole SWMb (11). In addition, a number of T cells responded to the fragment 1–55 (1:1,143). Although even approximate boundaries of an epitope(s) within the 1–55 region have not yet been defined, these results indicate that H-2^d–restricted T cells see at least three parts of the whole molecule of 153 amino acids, spreading out somewhere near the NH_2 terminal through 102–118 in the middle cyanogen bromide cleavage fragment to the COOH-terminal peptide 132–146.

In contrast, most of the H-2^k–restricted T cells responded to the fragment 1–55 (1:1,927 of a total of 1:1,708), and few, if any, could be stimulated by peptide 102–118 or 132–146 (<1:60,000; Fig. 4). Furthermore, no detectable H-2^k–restricted T cells responded to SWMb on B10.A(4R) spleen cells that bear only I-A^k and not the I-E^k molecule (Fig. 5, *right*). Other accessory functions of the B10.A(4R) spleen cells were intact, as T cells could be stimulated well with Con A on the 4R spleen cells (data not shown). Thus, their restriction was limited to I-E^k.

Both I-A^d and I-E^d restrictions were seen in H-2^d–restricted response. Almost all of the T cell lines that responded to peptide 102–118 could be stimulated by the same peptide on B10.GD spleen cells that have only I-A^d (Fig. 5, *left*; 1:673 compared with 1:645 in Fig. 3). The response to peptide 132–146 was not seen on B10.GD APC, confirming that the response is restricted to I-E^d (28). The majority of the T cells specific for the fragment 1–55 responded on B10.GD spleen cells (i.e., were A^d restricted). Although the first limiting dilution experiment did not have enough data on the restriction of response to fragment 1–55

TABLE I
Summary of Clonal Frequencies

Exp.	Restriction	Antigen				
		SWMb	1–55	59–80	102–118	132–146
1	H-2^d	1:475	1:143		1:645	1:4,876
	I-A^d	1:480			1:673	<1:63,400
	I-E^d					1:4,876
	H-2^k	1:1,708	1:1,927		<1:63,400	<1:63,400
	I-A^k	<1:63,400	<1:63,400		<1:63,400	<1:63,400
	I-E^k	1:1,708	1:1,927		<1:63,400	<1:63,400
2	H-2^d	1:398	1:2,390	1:10,565	1:393	1:7,015
	I-A^d	1:398	1:2,390	1:10,565	1:449	<1:14,400
	I-E^d					1:7,015
	H-2^k	1:1,018	1:1,434	1:999	<1:30,000	<1:30,000
	I-A^k	<1:30,000	<1:30,000	1:1,818	<1:30,000	<1:30,000
	I-E^k	1:1,018	1:1,434		<1:30,000	<1:30,000

because of the short supply of the fragment, the result was confirmed in the second limiting dilution experiment (Table I).

To confirm these results, the limiting dilution analysis was repeated on a slightly smaller scale with a new group of F_1 mice. The results, summarized in Table I, showed that (*a*) the majority of H-2^d–restricted T cells was 102–118 specific and I-A^d restricted; (*b*) the response to peptide 132–146 was not seen on I-E^{d-} B10.GD APC; (*c*) none of the H-2^k–restricted T cells responded to peptide 102–118 or 132–146; and (*d*) whole SWMb and fragment 1–55 did not stimulate H-2^k T cells on I-E^{k-} B10.A(4R) APC. In addition, a new peptide 59–80 that covers one of the amphipathic helical regions of SWMb (36, 37) was synthesized (Cease K. B., S. J. Brett, M. Kojma, and J. A. Berzofsky, manuscript in preparation). This peptide was shown to stimulate SWMb-primed lymph node T cells from H-2^k mice and was identified independently by Livingstone and Fathman (38), so the new peptide was also tested for its ability to stimulate the second set of the limiting dilution cell lines.

Strikingly, the peptide 59–80 stimulated almost all of the H-2^k–restricted cell lines very well. The majority of these T cells responded to both peptide 59–80 and fragment 1–55. The frequencies from Poisson analysis indicate that there must be dual specificities or crossreaction between 59–80 and 1–55. We have some preliminary evidence for possible crossreaction (unpublished observations), but for purposes of the issues addressed in the current paper, the exact fine specificities of these cells are not critical. Further work with additional peptides will be required. In contrast to the H-2^k–restricted cells, 59–80–reactive H-2^d–restricted T cells were very rare, and only one cell line responded to it.

These results indicate that low responder H-2^k–restricted T cells have a limited repertoire to see myoglobin only in the 1–55 region or 59–80 region with I-E^k restriction. High responder H-2^d–restricted T cells have a much broader spectrum of fine specificities using both I-A^d and I-E^d restriction elements and, in particular, respond to the immunodominant antigenic site around Glu 109 in the context of I-A^d.

Response of H-2^s– or H-2^b–restricted T Cells to the Peptides. Besides H-2^d and

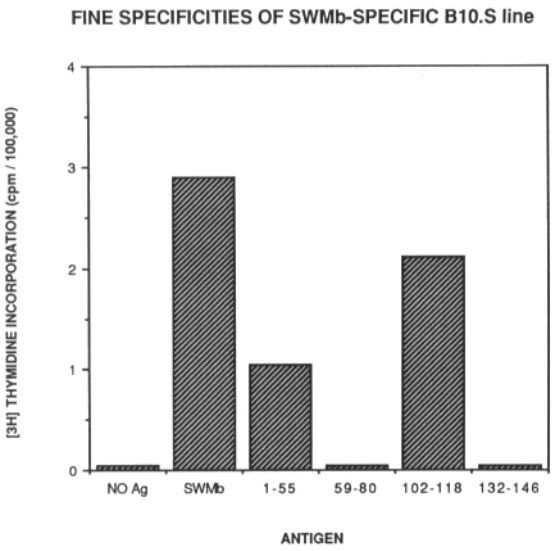

FIGURE 6. Fine specificities of SWMb-specific B10.S T cell line. The SWMb-specific B10.S T cell line was made by the same method as the (B10.BR × B10.D2)F_1 T cell lines but without limiting dilution. After four rounds of stimulation cycles, the polyclonal T cell line was tested for proliferation to SWMb, fragment 1–55, and peptides 59–80, 102–118, and 132–146. For details, see Materials and Methods.

H-2^k, H-2^s is another high responder and H-2^b is another low responder to SWMb (4, 11, 12). To extend the information on the mechanism of high and low responsiveness, SWMb-specific short-term B10 and B10.S T cell lines were tested for their response to the synthetic peptides and the fragment 1–55. The T cell lines were stimulated and rested for more than five rounds to reduce background response and to enrich specific T cells. The B10.S line showed a strong response to peptide 102–118 and fragment 1–55 but did not respond to either peptide 59–80 or 132–146 (Fig. 6). Since H-2^s has only I-A molecules, again the dominant response in this other high responder was toward peptide 102–118 plus the I-A^s restriction element, as suggested from the crossreactivities for whale myoglobin evolutionary variants (26).

The B10 line, in turn, showed a strong response only to fragment 1–55 and no response to peptide 59–80 or 132–146 (Fig. 7). A very weak response could be detected to peptide 102–118 in the whole population, but most of the cell line consisted of 1–55-specific T cell clones, and no 102–118-specific clones could be isolated. The response to peptide 102–118 was not observed in fresh lymph node T cells (data not shown), so repeated stimulations may have enriched the 102–118-specific T cells to a detectable level. However, the response was still low. It might be that (*a*) peptide 102–118 did not cover required amino acid sequences and only caused weak stimulation to the H-2^b–restricted T cells; (*b*) SWMb was never processed to show epitope(s) of peptide 102–118 and T cells specific to this peptide survived with bystander help; or (*c*) a suppression mechanism reduces response to peptide 102–118. Whatever the case is, response to peptide 102–118 was marginal in I-A^b–restricted T cells.

Thus, the overall picture appears to be that the high responder H-2^d– and H-2^s–restricted T cells could respond to the immunodominant peptide 102–118 in the context of an I-A molecule, and the low responder H-2^k– and H-2^b–restricted T cells lack this dominant response to peptide 102–118 plus an I-A restriction element. Both low responders appear to have a more limited T cell repertoire

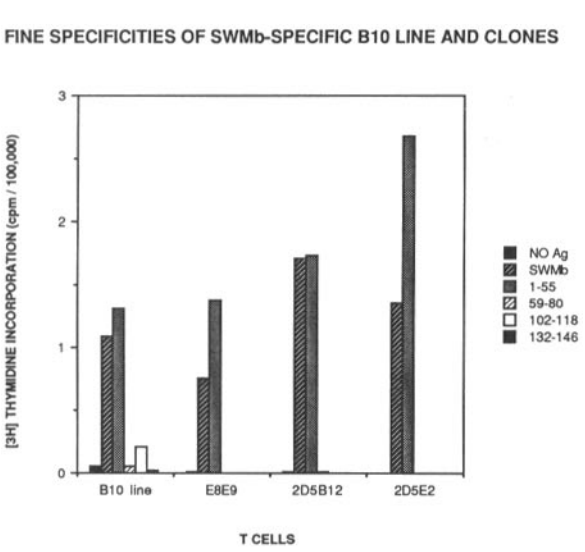

FIGURE 7. Fine specificities of SWMb-specific B10 T cell lines and clones. SWMb-specific B10 T cell line was prepared by the same method as the B10.S T cell line. After six rounds of stimulation cycles when T cells started to expand, they were cloned out at a dilution of 0.3–0.5 cells per well, twice. Clones E8E9, 2D5B12, and 2D5E2 were tested along with the B10 line for proliferation to SWMb, fragment 1–55, and peptides 59–80, 102–118, and 132–146.

focused on the region 1–55 (or the crossreactive 59–80) and use a single restriction element (I-E^k for H-2^k, and I-A^b for H-2^b).

Discussion

A long-term limiting dilution Poisson analysis was performed to approach the question of why a high responder is high and a low responder is low in response to a whole complex protein antigen, in this case, SWMb. (B10.BR [low] × B10.D2 [high])F_1 T cells, after limiting dilution, were repeatedly stimulated, expanded, and maintained on high or low responder APCs as limiting dilution cell lines. Their reactivities to fragment 1–55, peptide 102–118, or peptide 132–146, along with their *Ir* restrictions, were scored as a function of the original limiting dilution cell numbers, and the frequencies of T cells of each fine specificity were calculated according to a Poisson distribution. The results showed that (*a*) there was only a fewfold difference in frequencies between H-2^d (B10.D2)–restricted and H-2^k (B10.BR)–restricted F_1 T cells specific to SWMb; (*b*) H-2^d–restricted T cells had a broader spectrum of fine specificities using both I-A^d and I-E^d restriction elements, especially focused on an immunodominant epitope defined by peptide 102–118 in the context of I-A^d; (*c*) most of the I-E^d–restricted T cells were specific for peptide 132–146, and the frequency of such cells was ~10% of the total H-2^d–restricted SWMb-specific T cells; (*d*) low responder H-2^k–restricted T cells had a limited repertoire for SWMb confined to 1–55 or 59–80 in association with I-E^k and lacked a response to the immunodominant site 102–118 in the context of an I-A molecule; (*e*) a comparable number of H-2^d– and H-2^k–restricted T cells were stimulated with fragment 1–55; and (*f*) the other high responder strain (H-2^s), which expresses only an I-A molecule, also showed a strong response to peptide 102–118 and to fragment 1–55, whereas in the other low responder strain B10 (H-2^b, I-E negative), T cells were mostly specific for 1–55 and only marginally responsive to peptide 102–118 (which did not stimulate a lymph node T cell response at all). Thus, we conclude (*a*) that there

was a consistent difference between the number of cells responding to the immunodominant epitope 102–118 in association with I-A and the numbers responding to other minor epitopes; and (*b*) that high responsiveness correlated with the response to this immunodominant epitope, whereas low responsiveness correlated with a lack of response to this epitope, even though other epitopes were seen.

The finding of only a fewfold difference in the frequencies of SWMb-specific T cells between high and low responder MHC restriction may be due (in part) to the first round stimulation and the rest of F_1 T cells on F_1 APC in vitro. Also, since we could not see a primary SWMb-specific response in vitro, it was obligatory to prime in vivo in the first place, so any difference in efficiency of F_1 APC might have already produced an effect even before in vitro culture on F_1 APC. To test the possibility that stimulating F_1 APCs would favor only one of the parental MHC-restricted T cells to expand, an F_1 APC titration was carried out. The result did not show any significant difference in the ability of F_1 APC to stimulate groups of randomly chosen cell lines of both MHC restriction types, although some variation from cell line to cell line was seen within each group. Thus, if the lines that grew at all in vitro on parental presenting cells were representative, we could not find evidence for any selective pressure due to preferential sensitivity of the different restriction types for growth with F_1-presenting cells in vivo or during the first round of stimulation in vitro. In addition, when SWMb-primed F_1 lymph node T cells were tested with the adjuvant component PPD as a control, the H-2^d–restricted response was barely 1.5-fold higher than the H-2^k–restricted response. This difference is much less than that for myoglobin and is not likely to be the general case for all antigens, because a low responder to SWMb may be a high responder to other antigens.

These results suggest that the low response of F_1 T cells on H-2^k–presenting cells may be due to the failure to see myoglobin plus I-A^k, in particular the major site around Glu 109, in contrast to the dominant response of high responder H-2^d and H-2^s strains focused on the I-A molecule and the site around residue 109. I-A^+ I-E^- low responder B10 mice, which are forced to use an I-A restriction element, also did not focus on 102–118. High response may be based on the additional response of T cells that are specific for 102–118 plus an I-A molecule, over and above the response to fragment 1–55 by other T cells, which were common in both high and low responder strains. We do not have strains that are negative to fragment 1–55 and so cannot determine whether strains that respond to peptide 102–118 and not to fragment 1–55 would still be high responders or not. Because the difference in the frequencies of SWMb-specific F_1 T cells between high and low responder MHC restrictions was only a fewfold, it seems to be unlikely that precursor T cell frequencies alone determine high responsiveness or low responsiveness, unless there is a threshold in the number of T cells to be expanded within the few fold differences. One way to study the importance of 102–118-specific T cells and the influence of 1–55-specific T cells may be to make a high responder mouse tolerant to the 102–118 peptide or to fragment 1–55. This raises quite an interesting problem of whether tolerance to a major antigenic epitope would result in low responsiveness to a certain antigen or not.

From these results, what can we say about the mechanism of *Ir* gene control of immune responses to natural whole protein antigens as might be encountered in nature or in vaccines? The failure of H-2^k–restricted T cells to respond to the immunodominant site around residues 102–118 in association with A^k could still be due to either of the two just-mentioned mechanisms; i.e., 102–118 could fail to bind to A^k or the T cells could be absent from the repertoire for other reasons. What is newly emphasized by this study is that although two strains may have comparable responses to other epitopes, such as ones in the 1–55 region, the difference between a high responder and a low responder can depend not just on the number of epitopes seen but on the presence or absence of a response to a single immunodominant site. This can occur because the response to that site stands out in magnitude greater than the response to any of the other sites to which both strains respond. Why should the response to one site so predominate; i.e., be immunodominant? Why does the response to other sites not make up for the lack of a response to this one in strains that do not see it? It does not appear to be simply due to a greater efficiency of presentation by F_1-presenting cells, at least as detectable in a titration of presenting cell numbers as in Fig. 2. Since whole myoglobin was used in these titrations, but the T cells were specific for different epitopes, it does not appear to be due to a greater efficiency of processing of the antigen to produce one peptide vs. the other. Similarly, there is not a striking difference in peptide dose–response curves to account for the differences seen. If simply the affinity of each peptide for its respective MHC molecule made the difference, then one would expect that the immunodominant site would stimulate at a lower concentration than a nonimmunodominant site. One possibility is that the repertoire for the immunodominant site is inherently larger. While this may relate to the repertoire development in the responding individual, it is also possible that it depends on certain properties intrinsic to the antigen. We know that there are multiple ways of seeing the 102–118 peptide by different T cell clones (33), and the same observation applies to other immunodominant sites of other antigens, such as lysozyme (29, 39, 40), OVA (41), and cytochrome *c* (42). If an immunodominant site is actually a cluster of overlapping epitopes, the repertoire that can see that region may be greatly increased. An intrinsic structural property of such sites, such as but not limited to amphipathicity (36, 37, 43, 44) or α-helicity (42, 43, 45, 46), may make them inherently more likely to be seen by T cells and therefore to elicit a broader repertoire beyond any differences in affinity for a particular MHC molecule or any single T cell receptor. Certainly these other factors are important, but these intrinsic features of certain sites would appear to operate on another level, on which these other selective factors are then superimposed. An additional possibility is that the immunodominant epitopes may be the ones that are less downregulated by (or less susceptible to) suppressor or other regulatory mechanisms. The T cells specific for minor epitopes may be kept from expanding to immunodominant levels by such regulatory mechanisms. The aggregate of all these effects, we suggest, leads to immunodominance and the quantitative difference between a high responder and a low responder.

Summary

To approach the mechanism that determines *Ir* gene–controlled high or low responsiveness to whole proteins, such as sperm whale myoglobin (SWMb), we compared the repertoires of high and low responder haplotype-restricted T cells for different myoglobin epitopes by limiting dilution frequency analysis. Poisson analysis was performed using long-term limiting dilution cell lines of (B10.BR [low] × B10.D2 [high])F_1 T cells maintained on high or low responder APCs. The cell lines were tested with SWMb peptides and fragments for T cell repertoire fine specificities and Ia restrictions. The frequency of SWMb-specific F_1 T cells responsive on B10.BR (H-2^k) APCs was 2.5–3.6-fold lower than on B10.D2 (H-2^d) APCs. Strikingly, all of the H-2^k–restricted T cells used I-E^k as a restriction element, whereas both I-A^d– and I-E^d–restricted T cells were found among the H-2^d–restricted lines. The I-A^d–restricted T cells were dominant, and the majority was specific for the synthetic peptide 102–118. T cells specific for peptide 132–146, dominant in association with I-E^d, were less frequent. However, no detectable H-2^k–restricted T cells were specific for either of these peptides, but instead they were specific for fragment 1–55 or peptide 59–80. Fragment 1–55 also stimulated a similar number of H-2^d–restricted T cells. Therefore, the low response of F_1 T cells on H-2^k–presenting cells may be due to the failure to see myoglobin plus I-A^k, in particular the immunodominant site around Glu 109, in contrast to the dominant response of high responder mice (both H-2^d and H-2^s) focused on the I-A molecule and the site around residue Glu 109. The I-E^- low responder B10 strain also failed to respond to peptide 102–118, supporting the idea that the low responder status results from a limited repertoire lacking response to 102–118 plus I-A. In those strains that respond to the immunodominant site 102–118, the frequency of T cells in the repertoire specific for this site was always considerably greater than that for other sites. These results suggest that there is an important difference between immunodominant epitopes and minor epitopes and that *Ir* gene–controlled low responsiveness to a natural whole protein may be due primarily to the failure to respond to a single immunodominant site, even though a number of other epitopes can be recognized.

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