

DIFFERENTIAL MAJOR HISTOCOMPATIBILITY  
COMPLEX-RELATED ACTIVATION  
OF IDIOTYPIC SUPPRESSOR T CELLS  
Suppressor T Cells Cross-Reactive to Two Distantly  
Related Lysozymes Are Not Induced by One of Them\*

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T cells exert a critical role in the regulation of the antibody response (1). Subpopulations of suppressor and helper T cells are phenotypically distinguishable (2), and the result of their interactions has been shown to be a major factor in the regulation and fine tuning of immune responsiveness (3, 4). The induction and the activity of suppressor and helper T cells are strongly influenced by immune-response (Ir)<sup>1</sup> genes linked to the major histocompatibility complex (MHC), H-2 in the mouse (5).

Previous studies in our laboratory have demonstrated that differential responsiveness to various lysozymes is controlled by H-2-linked Ir genes (6). Lysozymes are tight globular proteins with a molecular weight close to 14,300. There is a substantial family of sequenced avian lysozymes (7) which show strong antibody (and T helper) cross-reactivity. Mammalian lysozymes, such as human (HUL) retain the same tertiary structure as avian lysozymes but are grossly changed with regard to surface amino acids and show little antibody cross-reactivity. Mice of H-2<sup>b</sup> and H-2<sup>s</sup> haplotypes are genetically nonresponsive, whereas mice of other haplotypes are responders, after intraperitoneal immunization with hen egg-white lysozyme (HEL) in complete Freund's adjuvant (CFA), as judged by plaque-forming cell (PFC) assays (8), T cell proliferation (9), or serum antibody responses. This lack of response is associated with induction of HEL-specific suppressor T cells (10). Suppression is not induced by immunization with the closely related, immunogenic ring-necked pheasant egg-white

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*Abbreviations used in this paper:* BRBC, burro erythrocytes; CFA, complete Freund's adjuvant; GAT, L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup>; GRBC, goat erythrocytes; GT, L-glutamic acid<sup>50</sup>-L-tyrosine<sup>50</sup>; HEG, HEPES-buffered Eagles's minimum essential medium containing 0.25% gelatin; HEL, hen egg-white lysozyme; HGG, human gamma globulin; HUL, human lysozyme; HuRBC, human erythrocytes; αIdX-HEL, anti-idiotypic against B10.A anti-HEL; MBSA, methylated bovine serum albumin; MHC, major histocompatibility locus; N-C, a.a. 1-17:cys6-cys127:120-129 of the HEL; PFC, plaque-forming cells; RBC, erythrocytes; REL, ring-necked pheasant egg-white lysozyme; SRBC, sheep erythrocytes.

lysozyme (REL): likewise, HEL-specific suppressor T cells do not affect the response to REL. The lack of REL recognition by HEL-specific suppressor T cells has been explained by the presence in HEL of a suppressor determinant, which is absent from REL. This suppressor determinant is included in the N-terminal, C-terminal peptide a.a. 1-17:cys6-cys127:120-129 of the HEL molecule (N-C) (11, 12). Nonimmunogenic lysozymes (e.g. HEL and HUL) have phenylalanine at residue 3, whereas the presence of tyrosine at this position, as in REL, Japanese quail egg-white lysozyme, or turkey egg-white lysozyme, seems to correlate with immunogenicity in C57BL/10Sn (B10) mice. HUL, unlike HEL, is not responded to in H-2<sup>q</sup>, H-2<sup>d</sup>, or H-2<sup>r</sup> mice (13). As with HEL, H-2<sup>b</sup> mice respond to HUL-coupled erythrocytes (RBC) and this response is suppressed by prior immunization with HUL-CFA. Although HEL and HUL differ at 52 amino acids out of 130 residues, they are similar in tertiary structure (14). Furthermore, both have phenylalanine at position 3 and a quite similar amino acid sequence at the N-terminus. It was therefore of interest to directly examine the suppressed *in vitro* response to HUL and compare the specificity of suppressors with those involved in the anti-HEL response.

Results presented in this study demonstrate that HEL and HUL priming generate suppressor T cells in B10 nonresponder mice that are highly cross-reactive in the induction and expression of suppressive activity. Suppressor cells induced by HEL and HUL share a common idiotypic determinant found on the majority of anti-HEL antibodies and on that small proportion of anti-HUL antibodies that are cross-reactive with HEL. The dissociation of H-2-linked Ir gene regulation of the *in vivo* responses to HEL and HUL, reported for the B10.Q (H-2<sup>q</sup>) strain, responsive to HEL but not to HUL, was further analyzed *in vitro*. In B10.Q mice, HEL-CFA priming induces helper cells, whereas HUL-CFA priming induces suppressor cells that are cross-reactive with HEL and are able to suppress HEL-specific helper cells. Although the required suppressor T cell for exerting cross-reactive suppression could be shown to exist in the B10.Q repertoire, these results indicate that Ir gene function is expressed at the level of antigen presentation.

### Materials and Methods

*Mice.* Female C57BL/Sn (B10) mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. B10.Q mice were bred and maintained from stock originally provided by Dr. J. Frelinger, Department of Microbiology, University of Southern California Medical School, Los Angeles, Calif. All mice were 2-3 mo old when immunized.

*Lysozymes.* HEL was purchased from Societa Prodotti Antibiotici, Milan, Italy. HUL was isolated, by Dr. D. Kipp, in our laboratory at the University of California at Los Angeles, from the urine of a patient with myelomonocytic leukemia by a modification of the procedure described by Canfield et al. (15). Briefly, the urine was adsorbed on a Bio-Rex 70 (Bio-Rad Laboratories, Richmond, Calif.) column equilibrated with 0.05 M sodium phosphate, pH 7.8. The column was then unpacked, washed extensively, repacked, and the HUL eluted with 0.5 M ammonium carbonate, pH 7.8. After lyophilization, HUL was further purified on CM-Sephadex (Pharmacia, Uppsala, Sweden) using a linear pH gradient of 0.05 M ammonium carbonate, from pH 7.0 to 9.0, followed by an ionic gradient from 0.05 to 0.5 M ammonium carbonate, pH 9.0. Purity for both lysozymes was established by column chromatography and slab gel electrophoretic analysis.

*Immunizations.* The immunization protocol consisted of a primary intraperitoneal injection of 100 µg lysozyme per mouse in 0.1 ml of saline emulsified with an equal part of CFA (Gibco Diagnostics, Gibco Invenex Div., Chagrin Falls, Ohio). Mice primed with a saline-CFA emulsion were used as control. 4 wk after immunization, mice were used for *in vitro* cultures, or

were challenged intravenously with  $10^8$  burro erythrocytes (BRBC) coupled with HEL or HUL (e.g., HEL-BRBC). Lysozyme-erythrocyte coupling procedures have been previously described (12).

*Assay of In Vivo PFC Response.* 6 d after lysozyme-BRBC challenge, mice were killed by cervical dislocation and the cell suspensions were gently teased from individual spleens and sequentially passed through coarse and fine mesh screens into cold HEPES-buffered Eagle's minimum essential medium (Gibco Diagnostics), containing 0.25% gelatin (HEG). The spleen cell suspensions were washed two times in HEG, resuspended in cold medium, and then assayed (16). As indicator cells, lysozymes coupled to goat erythrocytes (GRBC) or unconjugated BRBC were used at a final concentration of 1%. Guinea pig anti-mouse Ig developing serum was used at a final dilution of 1:400 to detect IgG PFC. Fresh guinea pig serum, absorbed with sheep erythrocytes (SRBC) was used as a source of complement (final dilution 1:64). Results are presented as the geometric mean of PFC/ $10^6$  nucleated spleen cells. A standard error coefficient was calculated from logarithmically transformed PFC and denotes a factor which multiplies and divides the mean to give the upper and lower limit of the standard error. IgG PFC refers to the number of PFC obtained with developing serum which inhibits, at the concentration used, 90% of the IgM PFC.

*In Vitro Cultures.* In vitro experiments were performed using a miniaturized two-chamber diffusion culture system recently developed in our laboratory (17). Culture medium was RPMI-1640 (Gibco Diagnostics) supplemented with 10% fetal calf serum (Flow Laboratories, Inc., Rockville, Md.), L-glutamine (Gibco Diagnostics),  $5 \times 10^{-5}$  M 2-mercaptoethanol and 10  $\mu$ g/ml gentamicin (Shering Corp., Kenilworth, N.J.). Briefly,  $2 \times 10^6$  spleen cells were cultured together with  $2 \times 10^6$  lysozyme-SRBC (Gibco Diagnostics) or human erythrocytes (HuRBC) (from a single donor) in 0.1 ml in the inner chamber, separated by a dialysis membrane from the reservoir in which 1 ml of medium was placed. At day 4 of culture, cells were harvested and tested for direct PFC against HEL-BRBC, BRBC, or SRBC, using the Cunningham and Szenberg (16) technique. Results are expressed as PFC per culture.

*Anti-Idiotype Preparation and Characterization.* Anti-idiotypic antisera were raised in guinea pigs by immunization with B10.A anti-HEL antibody populations. These antisera were rendered idiotypically specific by multiple passages over normal Ig adsorbents to remove antibodies against isotypic and allotypic determinants. This anti-idiotypic ( $\alpha$ IdX-HEL) reacts with the vast majority of anti-HEL antibodies produced by B10.A mice as well as all other strains tested, including B10 mice.  $\alpha$ IdX-HEL almost completely inhibits the interaction of HEL with anti-HEL antibodies, indicating recognition of a site spatially related to the antigen-binding site (18).

*Treatment of Spleen Cells with Antisera.* Anti-T serum was rabbit anti-mouse thymocyte serum (Microbiological Associates, Walkersville, Md.). To obtain a specific anti-T cell reagent, the serum was absorbed with XS-63 (a BALB/c, non-Ig-producing plasmacytoma), according to Kappler and Marrack (19). Anti-T serum absorbed twice with B10 thymocytes ( $10^8$  thymocytes/ml serum for 30 min at 4°C) was used as control. Anti-T serum was incubated for 30 min in an ice bath at 1:10 (final dilution) with  $2 \times 10^7$ /ml spleen cells, whereas  $\alpha$ IdX-HEL was used at 1:5 final dilution. Spleen cells were then washed once, resuspended in Low-Tox rabbit complement (Cedarlane Laboratories, London, Ontario, Canada) at a final dilution of 1:8, incubated for 30 min at 37°C, and then washed three times.

## Results

*Extensive Cross-Reaction at the Suppressor T Cell Level and Limited Cross-Reaction at the PFC Level is Observed, In Vivo, between HEL and HUL.* Five B10 mice per group were immunized with a saline-CFA emulsion, HEL-CFA (100  $\mu$ g/mouse), or HUL-CFA (100  $\mu$ g/mouse) and challenged 4 wk later with  $10^8$  HEL-BRBC or HUL-BRBC. 6 d after challenge, cells from individual spleens were assayed for IgG PFC using HEL-GRBC, HUL-GRBC, or BRBC as indicator cells. B10 mice, genetically nonresponsive to HEL (6), can generate a primary anti-HEL PFC response if HEL is coupled to an immunogenic carrier, such as RBC. However, if mice are preimmunized with HEL-

CFA and challenged with HEL-RBC, a specific suppression of the anti-HEL response is observed, although the anti-RBC response is unaffected (12). Data in Table I confirm and extend our previous results demonstrating that primary anti-lysozyme and anti-BRBC PFC responses were obtained after HEL-BRBC or HUL-BRBC injection. Preimmunization with either HEL-CFA or HUL-CFA induced suppression of the PFC responses to either lysozyme but did not affect the anti-BRBC response.

Very low cross-reactivity was found between HEL and HUL at the PFC level. Only 1% or less of the anti-HEL PFC were detected using HUL-GRBC, when HEL was the challenging lysozyme. In the converse situation, when HUL was the challenging lysozyme, 20% of the anti-HUL PFC were detected using HEL-GRBC as indicator cells. This low B cell cross-reactivity contrasts with relatively high cross-reactive suppression of anti-HEL or anti-HUL PFC responses after either HEL-CFA or HUL-CFA priming.

*HEL and HUL Show High Cross-Reactivity In Vitro at the Suppressor Cell Level.* Antibodies raised against HEL or HUL display very restricted cross-reactivity as judged by isoelectric focusing (20) or PFC assay (Table I). However, as indicated in Table I, HEL and HUL are able to induce cross-reactive suppression in vivo. To characterize the nature of the cross-reactivity, in vitro experiments were performed. B10 mice were primed with saline-CFA, HEL-CFA, or HUL-CFA. After 4 wk,  $2 \times 10^6$  spleen cells together with  $2 \times 10^6$  HEL-HuRBC or HUL-HuRBC were cultured in a miniaturized in vitro diffusion culture system. Direct anti-HEL or anti-HUL PFC/culture were assayed on day 4 of culture. Results in Table II demonstrate that in this system it was possible to obtain a primary response in vitro to HEL and HUL. However, in this as in other experiments, the primary anti-HUL response was only one-third or less of the primary anti-HEL response. After HEL or HUL priming, a comparable degree of suppression of either the anti-HEL or anti-HUL PFC response was observed. Coculture of 5% spleen cells from either HEL-CFA- or HUL-CFA-primed mice with 95% spleen cells from saline-CFA primed mice resulted in a very

TABLE I  
*HUL- and HEL-induced Suppressor Cells are Cross-Reactive in B10 Nonresponder Mice\**

Priming	Challenge	IgG PFC/ $10^6$ spleen cells			Suppression of anti-lysozyme response		Cross-reactivity PFC response $\ddagger$
		$\alpha$ HEL	$\alpha$ HUL	$\alpha$ BRBC	HEL	HUL	
					%		%
CFA	HEL-B	5,108 (1.36)	53 (1.23)	454 (1.10)	—		1
HEL-CFA	HEL-B	444 (3.25)	2 (1.07)	416 (1.74)	92		0.4
HUL-CFA	HEL-B	2,676 (1.81)	32 (3.15)	676 (1.36)	48		1
CFA	HUL-B	203 (1.01)	906 (1.29)	1,167 (1.62)		—	22
HUL-CFA	HUL-B	22 (2.43)	158 (1.82)	1,057 (1.74)		83	14
HEL-CFA	HUL-B	69 (1.90)	348 (1.02)	1,432 (1.14)		62	20

\* Five B10 mice per group. Mice were primed intraperitoneally with CFA or HEL-CFA (100  $\mu$ g) or HUL-CFA (100  $\mu$ g) and challenged 4 wk later with HEL-BRBC or HUL-BRBC ( $10^8$  intravenously). Spleen cells were assayed 6 d after challenge. Data are expressed as geometric mean PFC and (in parentheses) SE coefficient.

$\ddagger$  The percent cross-reactivity at the PFC level is calculated considering the response to the challenging lysozyme as 100% (e.g.,  $53/5,108 \times 100 = 1\%$  cross-reactivity).

TABLE II  
High HEL-HUL Cross-Reaction at the Suppressor Cell Level Is Observed In Vitro

Spleen cells in culture ( $\times 10^6$ ) from mice primed with:			Antigen in vitro	Anti-lysozyme direct PFC/culture	Suppression %
CFA	HEL-CFA	HUL-CFA			
2	—	—	HEL	728 $\pm$ 22	0
—	2	—	HEL	305 $\pm$ 52	59
—	—	2	HEL	253 $\pm$ 75	68
1.9	0.1	—	HEL	195 $\pm$ 15	74
1.9	—	0.1	HEL	253 $\pm$ 32	68
2	—	—	HUL	251 $\pm$ 56	0
—	2	—	HUL	51 $\pm$ 10	80
—	—	2	HUL	98 $\pm$ 32	61
1.9	0.1	—	HUL	88 $\pm$ 22	65
1.9	—	0.1	HUL	91 $\pm$ 43	64

B10 mice were primed with either saline-CFA, HEL-CFA (100  $\mu$ g), or HUL-CFA (100  $\mu$ g) 4 wk before culture.  $2 \times 10^6$  spleen cells and  $2 \times 10^6$  HEL-HuRBC were cultured in miniaturized diffusion culture chambers and separately assayed, on day 4 of culture, for direct anti-HEL PFC. PFC data are presented as arithmetic means and SE.

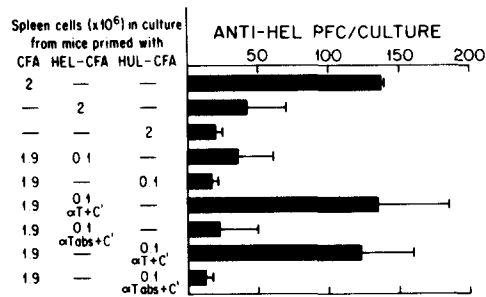


FIG. 1. The in vitro anti-HEL response can be suppressed by HEL- or HUL-induced anti-T sensitive cells. Mice were primed with saline-CFA, HEL-CFA, or HUL-CFA (100  $\mu$ g/mouse) intraperitoneally 4 wk before culture.  $2 \times 10^6$  spleen cells and  $2 \times 10^6$  HEL-SRBC were cultured in miniaturized diffusion chambers and assayed, on day 4 of culture, for direct anti-HEL PFC. Cell mixtures were 95% spleen cells from saline-CFA-primed mice and 5% spleen cells from HEL-CFA- or HUL-CFA-primed mice. Spleen cells from lysozyme-primed mice were sequentially incubated with rabbit anti-mouse thymocyte serum ( $\alpha$ T) at final dilution of 1:10 for 45 min at 4°C and rabbit complement (final dilution 1:8) for 30 min at 37°C;  $\alpha$ Tabs,  $\alpha$ T serum absorbed twice on an excess of B10 thymocytes. Data are presented as arithmetic means of PFC per culture and bars represent SE from triplicate cultures.

similar degree of suppression and indicates that HEL and HUL are highly cross-reactive both in the induction and in the expression of suppressive activity.

The presence of T cell markers on HEL- and HUL-induced suppressor cells was analyzed in the experiment reported in Fig. 1. As demonstrated in a previous experiment (Table II), spleen cells from B10 mice, primed 4 wk before culture with HEL-CFA or HUL-CFA were able to suppress the anti-HEL response of spleen cells from saline-CFA-primed mice. When HEL- or HUL-induced suppressor cells were treated with anti-T serum and complement, suppression of the primary anti-HEL response was abolished, whereas anti-T serum absorbed with thymocytes was ineffec-

TABLE III  
HEL- and HUL-induced Suppressor T Cells Share a Common Idiotype\*

Spleen cells ( $\times 10^6$ ) in culture from mice primed with:			Experiment 1			Experiment 2		
CFA	HEL-CFA	HUL-CFA	Anti-HEL PFC/culture	Suppression %	$P$ ‡	Anti-HEL PFC/culture	Suppression %	$P$
2	—	—	448 $\pm$ 74	0		137 $\pm$ 2	0	
—	2	—	86 $\pm$ 39	81	<0.02	43 $\pm$ 31	69	<0.05
—	—	2	201 $\pm$ 33	55	<0.05	18 $\pm$ 6	87	<0.01
1.9	0.1	—	113 $\pm$ 36	75	<0.02	36 $\pm$ 23	74	<0.05
1.9	—	0.1	170 $\pm$ 25	63	<0.05	16 $\pm$ 6	89	<0.01
1.9	0.1§ (NGPS+C')	—	163 $\pm$ 32	64	<0.05	8 $\pm$ 4	95	<0.01
1.9	0.1 (aid+C')	—	488 $\pm$ 60	0	NS	142 $\pm$ 37	0	NS
1.9	—	0.1 (NGPS+C')	118 $\pm$ 7	74	<0.02	26 $\pm$ 26	81	<0.05
1.9	—	0.1 (aid+C')	345 $\pm$ 100	23	NS	140 $\pm$ 70	0	NS

\* B10 mice were primed with either saline-CFA, HEL-CFA (100  $\mu$ g), or HUL-CFA (100  $\mu$ g) 4 wk before culture.  $2 \times 10^6$  spleen cells and  $2 \times 10^6$  HEL-SRBC were cultured in miniaturized diffusion culture chambers and separately assayed on day 4 of culture, for direct anti-HEL PFC. PFC data are presented as arithmetic means and SE from triplicate cultures.

§ Spleen cells were sequentially incubated with normal guinea pig serum (NGPS) (final dilution 1:10) or anti-idiotypic serum (aid) (final dilution 1:5) for 30 min at 4°C and rabbit complement (final dilution 1:8) for 30 min at 37°C.

‡  $P$  value calculated by Student's  $t$  test. NS, not significant ( $P > 0.1$ ).

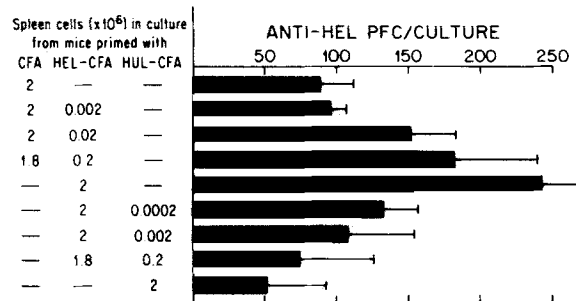


FIG. 2. Helper cell activity induced by HEL in B10.Q mice is suppressed by spleen cells from HUL-primed mice. Mice were primed with saline-CFA, HEL-CFA, or HUL-CFA (100  $\mu$ g/mouse) intraperitoneally 4 wk before culture.  $2 \times 10^6$  spleen cells and  $2 \times 10^6$  HEL-SRBC were cultured in miniaturized diffusion chambers and assayed, on day 4 of culture, for direct anti-HEL and anti-SRBC PFC. Data are presented as arithmetic means of PFC per culture and bars represent SE from quadruplicate cultures. Direct anti-SRBC PFC per culture, from top to bottom of the figure, were: 622  $\pm$  67, 575  $\pm$  59, 722  $\pm$  124, 687  $\pm$  119, 667  $\pm$  116, 622  $\pm$  96, 575  $\pm$  95, 612  $\pm$  59, 712  $\pm$  139.

tive. These results demonstrate that HEL-CFA or HUL-CFA priming of B10 mice induces a population of cross-reactive suppressor T cells. HEL- and HUL-induced suppressor T cells in B10 mice have been titrated in vitro (data not shown). About 35% suppression of the primary anti-HEL response was observed when as few as  $2 \times 10^2$  HEL-primed spleen cells were added to  $2 \times 10^6$  saline-CFA primed spleen cells and maximum suppression ( $\sim 60\%$ ) was reached upon addition of  $2 \times 10^4$  cells. Suppressor T cells from HUL-CFA immunized mice were equally effective in the generation of suppressive activity. Specificity of suppression was demonstrated by the similar values of anti-SRBC response obtained in each of the cultures.

*HEL- and HUL-induced Suppressor T Cells Share a Common Idiotype.* The great majority

of anti-HEL antibodies raised in mice bear a common or closely related idio type, designated IdX-HEL. We have recently demonstrated the presence of this cross-reactive idio type on HEL-induced suppressor T cells in the B10 strain (18). Antigen-specific suppression was eliminated by treatment of the suppressor population with anti-idiotypic serum and complement. Anti-HUL antibodies cross-reactive with HEL (5-20% of the total anti-HUL response) bear idiotypic determinant(s) found on the majority of anti-HEL antibodies (20). Therefore, the possibility of expression of common idiotypic determinants on HEL- or HUL-induced suppressor T cells was examined (Table III). As before,  $2 \times 10^6$  spleen cells from saline-CFA-, HEL-CFA-, or HUL-CFA-primed B10 mice were cultured with  $2 \times 10^6$  HEL-SRBC and the anti-HEL PFC response assayed on day 4 of culture. The anti-HEL response was suppressed in spleen cell cultures from HEL-CFA- or HUL-CFA-primed mice; cultures containing 5% of spleen cells from mice primed with either lysozyme, mixed with 95% spleen cells from saline-CFA-primed mice, exhibited the same degree of suppression. Treatment of the HEL- or HUL-induced suppressive cell population with  $\alpha$ IdX-HEL (raised in guinea pigs against B10.A anti-HEL) and complement eliminated suppression of the anti-HEL primary response, whereas treatment with normal guinea pig serum and complement was ineffective. These results confirm the presence of IdX-HEL determinants on suppressor T cells and indicate that HEL- and HUL-induced suppressor T cells share a common idio type.

*The Activity of HEL-induced Helper Cells in B10.Q Mice is Suppressed by Spleen Cells from HUL-primed Mice.* B10.Q mice (H-2<sup>q</sup>) are responsive in vivo to HEL but do not respond to HUL. An in vitro titration of the activity of HEL-CFA- and HUL-CFA-primed spleen cells is presented in Fig. 2.  $2 \times 10^6$  spleen cells from saline-CFA-, HEL-CFA-, or HUL-CFA-primed mice were cultured alone or mixed with graded numbers of spleen cells from other suspensions, in the presence of  $2 \times 10^6$  HEL-SRBC as antigen. Spleen cell density in all cultures was kept at  $2 \times 10^6/100 \mu\text{l}$  and triplicate cultures were individually assayed on day 4 of culture for direct anti-HEL and anti-SRBC PFC response. When an increasing proportion of spleen cells from HEL-CFA-primed mice was admixed with spleen cells from saline-CFA-primed mice, there was a gradual increase in anti-HEL response which reached a maximum of 243 PFC per culture for 100% HEL-primed cells. When these primed cells were mixed with as few as  $2 \times 10^2$  spleen cells from HUL-CFA-primed mice, the anti-HEL response was reduced by ~45% to 133 PFC. Increasing proportions of HUL-primed cells gave greater suppression which reached a maximum of ~80% (52 PFC). Specificity of the suppressive or helper effect was demonstrated by the similar values of anti-SRBC response obtained in all cell cultures. These results indicate that in B10.Q mice, HEL priming induces HEL-specific helper cells whereas HUL priming induces suppressor cells able to suppress HEL-specific helper cells.

### Discussion

We have demonstrated that in B10 mice, which are genetically nonresponsive to HEL or HUL, suppressor T cells are generated after intraperitoneal immunization with HEL-CFA or HUL-CFA. Despite very different amino acid composition (52 amino acid residues are different out of 130) HEL and HUL induce highly cross-reactive suppressor cell populations. However, in the B10.Q strain, where a similar cross-reactive suppressor cell can be shown to exist, its activity is not expressed after

HEL, but only after HUL immunization. This not only is an indication that the MHC influences activation of suppressor cells, but also that in the B10.Q the immunogenicity of HEL is not the result of a deletion in the suppressor cell repertoire.

*Specificity of the Cross-Reactive Suppressor Cell.* In B10 mice, HEL-specific suppressor T cells do not recognize the closely related immunogenic REL, which indicates that only a limited region on the HEL molecule, absent on REL, must account for the induction of suppression (10). The suppressor determinant of HEL for the B10 mouse is restricted to a limited portion of the molecule contained within the N-C peptide (12). Recent evidence indicates that suppressor T cells in B10 mice can also be induced by the purified N-terminal fragment of the N-C peptide. (M. Harvey et al. Manuscript in preparation.) All available evidence in the lysozyme system points to position 3 as critical in the composition of a determinant inducing suppressor cells in nonresponding mice. The nonimmunogenic lysozymes, such as HEL and HUL, have phenylalanine at this position rather than tyrosine, which is found in immunogenic lysozymes. The presence of a suppressive determinant common to HEL and HUL, despite the vast differences between these two lysozymes, can explain why suppressor T cells induced by HEL or HUL priming are highly cross-reactive. The N-terminal regions of HEL or HUL are identical in amino acid sequence for residues 1-9, except that glycine at residue 4 in HEL is replaced by glutamic acid in HUL. Still open is the possibility that HUL-induced suppression of anti-HUL responsiveness may in addition involve other suppressive determinants able to generate HUL-specific suppressor T cells which are non-cross-reactive with HEL.

We have found common idiotypic determinants present on HEL-specific suppressor T cells and on the large majority of anti-HEL antibodies produced in the secondary response (20). The sensitivity of HUL-induced, HEL-cross-reactive suppressor T cells to anti-idiotypic serum, raised against anti-HEL antibodies, suggests that HEL and HUL stimulate the same population of suppressor T cells in B10 mice. The close similarity between HEL and HUL in the N-terminal region, which contains the suppressive determinant on HEL, is consistent with the idea that the idiotypic receptor on the cross-reactive suppressor T cells recognizes a common determinant present near the N-terminus of both molecules.

Cross-reactivity at the suppressor T cell level has been demonstrated for the random copolymers L-glutamic acid<sup>50</sup>-L-tyrosine<sup>50</sup> (GT) and L-glutamic acid<sup>60</sup>-L-alanine<sup>30</sup>-L-tyrosine<sup>10</sup> (GAT) which, when injected in mice bearing nonresponder haplotypes, suppress the response to these copolymers complexed with methylated bovine serum albumin (MBSA). In SJL nonresponder mice, GT-induced suppressor cells are able to suppress both GT-MBSA and GAT-MBSA responses (21). GT and GAT, however, are highly cross-reactive at the antibody level. A pattern of cross-reactivity closer to the one observed between HEL and HUL has been reported in studies of tolerance to gamma globulins. Basten et al. (22) have demonstrated that suppressor T cells are generated during induction of tolerance to human gamma globulin (HGG). HGG-induced suppressor cells were found able to cross-suppress the antibody response to bovine gamma globulin which is only weakly cross-reactive with HGG at the B cell level (23). Moreover, Ruben et al. (24) previously demonstrated that mice rendered unresponsive to HGG show a marked tolerance to porcine gamma globulin and equine gamma globulin, in spite of minimal cross-reactivity at the PFC level among these antigens.



An implication of the present and other (25, 26) studies that have used protein antigens to examine the fine specificity of T and B cells is that different structural requirements exist for the activation of these two cell populations. B cells and antibody specificities have been related to the recognition of conformationally determined epitopes on the antigen surface whereas T cells seem to recognize sequential determinants inscribed in the primary amino acid structure. Similarities in the primary amino acid sequences of HEL and HUL in a small region containing a suppressor determinant could explain the convergence of their suppressive "libraries", without significant antibody cross-reactivity.

*Ir Gene Control at the Level of Activation of a Suppressor Cell.* B10.Q (H-2<sup>q</sup>) mice produce anti-HEL PFC but are not responsive to HUL. We have shown that, in this strain, T suppressor cells are demonstrable after HUL priming and helper cells after HEL priming. Interestingly, although HEL could not induce detectable suppressor cells, HUL-induced suppressor cells were able to fully suppress HEL-induced helper cell activity. There was no indication that the response to HEL was caused by the generation of helper cells that were resistant to suppression. Furthermore, it appears HUL-induced suppressor cells in B10.Q mice bear the cross-reactive idiotype and are completely analogous to the HEL-HUL cross-reactive suppressor cells in the B10 strain. (D. Rozycka-Jackson and L. Adorini. Unpublished observations.)

These results imply that failure to activate a potentially functional suppressor cell present in the repertoire allows responsiveness to HEL in B10.Q mice. It is likely that H-2<sup>q</sup> antigen presenting cells process HEL and HUL differently because of their differences in amino acid sequence. However, unlike the H-2<sup>b</sup> in which HUL and HEL can each activate the IdX-bearing suppressor cell, in the B10.Q strain, the suppressive determinant on HEL, but not on HUL, may be obscured, compromising its recognition by suppressor precursor cells.

This may represent a common occurrence in the responsiveness to the array of epitopes on multideterminant antigens in the sense that a potentially expressible cell type remains quiescent owing to a lack of appropriate epitope presentation. One of the manifestations of Ir gene control appears to be the presentation of epitopes to functionally different T cell subpopulations by MHC molecules (27, 28). In both the anti-GAT and anti-HEL antibody responses, Ir gene-controlled activation of helper or suppressor T cells seems to result from selective stimulation by antigen-presenting cells of either T cell subset, which drives the system to predominant helper or suppressor activity. Induction in H-2<sup>q</sup> mice of helper cells by HEL and of suppressor cells only by HUL, despite the existence of an HEL-HUL cross-reactive suppressive system, can similarly be explained by differential interaction between each lysozyme and the antigen-presenting devices controlled by H-2-linked Ir genes, leading to predominant activation of a single T cell subpopulation.

### Summary

B10 (H-2<sup>b</sup>) mice are genetic nonresponders to hen egg-white lysozyme (HEL) and the distantly related human lysozyme (HUL). However, anti-HEL or anti-HUL primary antibody responses *in vivo* or *in vitro* can be obtained in B10 mice by immunization with the appropriate lysozyme coupled to erythrocytes. T cells able to suppress either anti-lysozyme plaque-forming cells (PFC) response are induced in B10 mice after immunization with HEL-complete Freund's adjuvant (CFA) or HUL-

CFA. This high cross-reactivity of HEL and HUL in the induction and the expression of suppressive activity is in marked contrast to their very low cross-reactivity at the PFC level.

These results suggest that either HEL or HUL can stimulate a suppressor T cell which recognizes a particular epitope present on both lysozymes. Suppressor cells induced by HEL or HUL bear the same predominant idiootype found on the majority of anti-HEL antibodies, and on the small proportion of anti-HUL antibodies cross-reactive with HEL.

B10.Q (H-2<sup>q</sup>) mice are responders in vivo to HEL-CFA, but not to HUL-CFA. In contrast to B10, HEL-CFA priming in B10.Q mice induces helper cells whereas HUL-CFA priming induces suppressor cells. These suppressor cells are cross-reactive with HEL and are fully able to suppress HEL-specific helper cells. The presence of HEL-specific suppressor cell precursors in B10.Q mice which are not activated by HEL, seems to implicate differential choice by the antigen presenting system as a basis for Ir gene control, rather than the absence of a regulatory cell type from the T cell repertoire.

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