PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **182**, 6–10 (1986)

Cellular Basis of Genetic Nonresponsiveness to Egg White Lysozyme (42301)

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Abstract. Utilizing radiation chimeras, we have investigated the cellular level at which the low immunological responsiveness to egg white lysozyme C57Bl/10 mice is expressed. Both NR → F1 (responder) and F1 → NR combinations were assessed. The results demonstrate that C57Bl/10 bone marrow can give rise to hen egg white lysozyme responsive cells, but this response requires that the antigen be presented by cells derived from high responder animals.

The immune response of mice to hen egg white lysozyme (HEL) is under H2-linked IR gene regulation. Mice of the $H2^b$ and $H2^s$ haplotypes mount only weak splenic plaque forming cell (PFC) responses when challenged with this antigen (1, 2). Nevertheless, several factors suggest that these animals harbor "normal" anti-HEL response capability. Thus, C57Bl/10 mice are able to mount primary anti-HEL PFC responses equivalent to those of high responder strains following challenge with a HEL–lipopolysaccharide (LPS) complex (3), and peripheral lymph nodes of low and high responder strains exhibit equivalent anti-HEL T cell responses upon direct immunization with HEL in Freund's complete adjuvant (FCA) (4, 5). Furthermore, the low response trait is not absolute, with up to 10% of C57Bl/10 mice being able to respond vigorously to any given challenge with HEL (1).

One outcome of the exposure of $H2^b$ mice to HEL is the induction of suppressor T cells with specificity for a single epitope on the HEL molecule (6, 7). It has been suggested that low responsiveness is a consequence of these suppressor cells (5). Others have suggested that a deficiency exists in these mice at the level of helper cell recognition of antigen presenting cell (APC) bound antigen (8). The latter investigators, utilizing radiation chimeras, were unable to demonstrate HEL specific $H2^b$ helper cell activation regardless of the source of the APC function. In the present communication, we present evidence which suggests that a response defect does indeed lie at the level of helper cell–APC interaction, but that it is the context in which the antigen is presented which dictates the response characteristic.

Materials and Methods. *Animals.* A/J and C57Bl/10 SgSn (B10) mice were purchased from Jackson Laboratories, Bar Harbor, Maine. C57Bl/10 SgSn♀ × A/J♂ F1 mice were bred in our own facility utilizing the Jackson derived strains as breeding stock. All animals were 10 or more weeks old at the inception of any given experiment. Chimeras were established by iv transfer of 3.5×10^7 anti-T cell treated (9) bone marrow cells into 900 R X-irradiated recipients. All recipients were maintained on acidified (0.01 *M* HCl) chlorinated (0.2% Clorox) water for 6 weeks postirradiation. Additionally, the recipients were injected intraperitoneally every other day with 1.0 ml of RPMI 1640 (GIBCO) containing 10 μg/ml gentamicin, for a total of 3 weeks postirradiation. Following this protocol resulted in a recipient survival of 95% 4 months postirradiation. The chimerism of the recipients was monitored by H2 typing of the peripheral blood mononuclear fraction. We utilized the cytofluorochromasia adaptation (10) of the microdroplet method (11) for this assay. Anti-D^d (B10 × LP.RIII)F1 anti-B10.A(5R) and anti-K^b I^b (A × B10.D2)F1 anti-B10.A(5R) were obtained from the NIAID serum bank. EL-4 absorbed rabbit serum was utilized as the source of complement. All of the chimeras reported here contained exclusively the white blood cell type of the bone marrow donor as determined by this assay.

Antigens. Lysozyme was obtained from

0037-9727/86 $1.50

Miles Laboratories, Kankakee Illinois. Freund's complete adjuvant, containing *Mycobacterium tuberculosis* stain H37Ra, was obtained from Difco, Detroit, Michigan.

Cellular manipulations. Cell transfer procedures, immunizations, and antibody-forming cell assays were all as previously described (9, 12). IgG comprised the major or only component of the response in all of the experiments presented here, and only IgG responses are recorded.

Results. Two types of experiments were performed using the reciprocal B10 → B_{10}AF1 and B_{10}AF1 → B10 chimeras. In the initial experiment, the chimeras, along with homologous bone marrow recipients and unmanipulated normal controls, were primarily immunized with 100 μg HEL in FCA and secondarily challenged with 100 μg aqueous HEL 1 month later. The splenic PFC responses of these animals, measured 5 days after the secondary challenge, are presented in Table I. As can be seen, the B10 → B_{10}AF1 chimeras behaved as do the bone marrow donors, with only one of nine animals yielding a strong response (group 6). In contrast the B_{10}AF1 → B10 chimeras behaved as the recipient (group 5). Thus, here also only one of the animals exhibited the high response characteristic.

At face value, these results might be interpreted to suggest that low responsiveness is dominant regardless of the chimeric combination. However, this type of experiemnt fails to distinguish the relative contributions of T cell and antigen presenting cell to the outcome. Both of these functions would be expected to derive from the bone marrow graft in long-term chimeras such as these. We therefore incorporated an additional manipulation into the experimental design in order to distinguish between the two. Thus, rather than prime the "chimeric" lymphocytes *in situ,* these cells were transferred into sublethally (600 R) irradiated secondary hosts. The cells were then both primed and secondarily challenged in this latter environment. The PFC responses of these animals are presented in Table II. As can be seen, here again low responsiveness seemed to dominate. However, some clear differences between the groups are apparent. Thus, for both B10 → F1 and F1 → B10 lymphocytes, a secondary F1 host was clearly more permissive of responsiveness than a secondary B10 host (groups 4 vs 5 and 6 vs 7). Furthermore, within the secondary F1 host, lymphocytes derived from the B10 → B_{10}AF1 chimeras were significantly more responsive ($P \leq 0.01$ disregarding the single high responder) than those from the B_{10}AF1 → B10 chimeras (group 6 vs 4). This suggested rather strongly that antigen presentation in the B10 context might play some role in the low response trait (see Discussion). We therefore sought to further clarify this point by performing the experiment presented in Table III. In this experiment, conventional HEL primed B_{10}AF1 lymphocytes were transferred into sublethally irradiated A/J, B10, or B_{10}AF1 hosts and secondarily challenged therein. As can be seen, although these cells could be induced to mount adoptive secondary responses in the two high responder hosts, they were an order of magnitude less responsive in the low responder (B10) environment.

TABLE I. RESPONSE OF B10 → B_{10}AF1 AND B_{10}AF1 → B10 CHIMERAS TO HEL

Donor/recipient combination	PFC/10 Spleen cells[a]	
B10 control	70 ± 17	(3)[b]
B_{10}AF1 control	1693 ± 120	(3)
B10 → B10	nil	(3)
B_{10}AF1 → B_{10}AF1	1911 ± 300	(3)
B_{10}AF1 → B10	392 ± 98	(3)
B10 → B_{10}AF1	2187	(1)
	nil	(8)
	2720	(1)

Note. Chimeras were established as described in the text. All animals were primed with 100 μg HEL in FCA and secondarily challenged with aqueous HEL 1 month later. PFC responses were assessed 5 days after secondary challenge.

[a] Mean ± SE.

[b] Number of animals.

Discussion. The experiments presented here were performed in order to gain some insight into the cellular level at which the low responsiveness to HEL of C57/B10 mice is expressed. Our results suggest that the defect in these animals is expressed at least in part at the level of antigen presentation. Thus, although F1 recipients which have been repopulated with B10 bone marrow are low responders, the lymphocytes of such animals

TABLE II. RESPONSE OF CHIMERA-DERIVED SPLEEN CELLS TO HEL IN SECONDARY ADOPTIVE HOSTS

Chimera/secondary host combination	PFC/10 Spleen cells[a]
B10 control	240 ± 10 (3)[b]
B_{10}AF1 control	3870 ± 130 (3)
(B_{10}AF1 → B_{10}AF1) → B_{10}AF1	2489 ± 765 (3)
(B_{10}AF1 → B10) → B_{10}AF1	103 ± 24 (4) 2133 (1)
(B_{10}AF1 → B10) → B10	nil (5)
(B10 → B_{10}AF1) → B_{10}AF1	654 ± 128 (4)
(B10 → B_{10}AF1) → B10	10 ± 9 (5)

Note. Following establishment of the chimeras, spleen cells from these animals were subsequently grafted into 600R X-irradiated secondary hosts as indicated. These animals were then primed with HEL in FCA and secondarily challenged with aqueous HEL. PFC responses were assessed 5 days after the secondary challenge.

[a] Mean ± SE.

[b] Number of animals.

were shown to respond significantly better in the presence of functional F1 APC. It is clear from this result that B10 bone marrow can give rise to HEL responsive cells. Since the B10 → B_{10}AF1 chimera would be expected to express only B10 APC function, it seemed reasonable to conclude that these cells failed to respond *in situ* because the antigen could not be appropriately "presented." This was confirmed by the experiment presented in Table III, wherein primed F1 lymphocytes were incapable of mounting a secondary response when presented with antigen in the B10 context.

Since "presentation" of antigen can be viewed as being different from "processing," it seems relevant to ask which might be deficient in the present case. Some insight can be gained from the finding that B_{10}AF1 → B10 lymphocytes were of low responder phenoype not only *in situ* but also when induced in conventional F1 hosts (Table I, group 5, Table II, group 4). Given that these cells would be expected to be restricted to antigen recognition in the B10 context, and since the HEL "processing" function of F1 APC is not in question, we must conclude that the problem resides either at the level of presentation of the antigen in the B10 context (e.g., inefficient association with class II elements) or at the level of helper cell recognition of the antigen in this context.

It has been suggested elsewhere (15) that the context in which antigen is presented may tilt the balance between helper and suppressor cells in the B10 response to HEL. Thus, antigen presentation in the low responder context might lead to preferential induction of suppression and associated nonresponsiveness. However, our findings are inconsistent with this idea. To begin with, HEL primed, high responder F1 lymphocytes are incapable of responding secondarily to HEL when it is presented in the low responder B10 context (Table III) whereas they respond quite nicely when the antigen is presented in either the A or F1 high responder context. It seems unlikely that a degree of suppression sufficient to abrogate the adoptive F1 response could be generated in the naive, irradiated B10 host during the short term of this experiment. One might argue that suppression is restricted to only those helper cells which recognize the antigen in the B10 context but this carries the corollary argument that the suppressed, B10 restricted helper cell population should be incapable of collaborating with B cells which express only the B10 context. That this is not the case is evident in the finding that B10 → B_{10}AF1 lymphocytes are capable of responding to HEL when stimulated in the presence of F1 APC (group 6, Table II). The responding B cells in this case are strictly of B10 origin. We thus find it difficult to invoke suppression to explain our findings.

TABLE III. ANTI-HEL RESPONSE OF HEL PRIMED B_{10}AF1 LYMPHOCYTES IN ADOPTIVE HOSTS

Recipient	B_{10}AF1 Spleen cells transferred	PFC/10 Spleen cells[a]
A/J	HEL primed	404 ± 95 (6)[b]
A/J	naive	11 ± 10 (4)
B10	HEL primed	33 ± 11 (6)
B10	naive	Nil (4)
B_{10}AF1	HEL primed	500 ± 136 (4)

Note. B_{10}AF1 mice were primed with HEL or saline in FCA. One month later, spleen cells from these animals were grafted into 600R X-irradiated naive recipients of the strain indicated. The latter were then challenged with aqueous HEL and their PFC responses assessed 6 days later.

[a] Mean ± SE.

[b] Number of animals.

The findings reported here differ significantly from those of an earlier study of chimeric mice in the B10 anti-HEL system (8). In those expeiments the investigators were unable to convert B10 lymphocytes to HEL responsiveness by allowing them to mature in F1 hosts. They therefore concluded that there is a central defect in the ability of the B10 strain to recognize HEL. This not only differs from our own conclusions but is at odds with a number of other studies which suggest that C57Bl/10 animals contain HEL responsive lymphocytes (1, 3–5). It should be pointed out that the majority of the findings in that previous study were subject to the same constraint as we encountered in our initial experiment (Table I). Thus, the chimeric lymphocytes were primed *in situ* and were therefore subject to the selective forces imparted by the available APC function. Although those investigators did, in one experiment, attempt to prime the chimeric lymphocytes in the presence of F1 APC function, this was accomplished by injecting the chimeras with irradiated F1 spleen cells just prior to priming. Unfortunately they were unable to control for whether the F1 APC function was successfully grafted. It is known that migration of grafted primed T cells to recipient lymphoid organs is deleteriously affected by *in vitro* irradiation of the cells (16), and it seems possible that the migration of reticuloendothelial cells might be similarly affected.

It may also be of relevance that those investigators utilized the recombinant B10.A(1R) as the responsive partner for their F1 cross. The anti-HEL response of these mice is significantly lower than that of the A/J utilized in the present experiments (17). C57Bl/10 mice are in general poor responders to a number of antigens (18–20), and use of the B10 congenic partner might have influenced the outcome of those experiments.

To summarize, C57Bl/10 lymphocytes, which normally respond poorly to HEL, can be converted to high responsiveness if allowed to both mature and respond in the presence of functional high responder antigen presentation function. The latter is critical; if these lymphocytes are presented with antigen in the low responder context they do not respond. We conclude that the anti-HEL response defect in these animals is at least in part due to a defect in the interaction between helper cells and antigen-presenting cells.

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Received May 7, 1985. P.S.E.B.M. 1986, Vol. 182.
Accepted January 9, 1986.