Brief Definitive Report

GENETICAL CONTROL OF B-CELL RESPONSES

IV. Inheritance of the Unresponsiveness to Lipopolysaccharides*

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Two parameters of the unresponsiveness to lipopolysaccharides from *Escherichia coli* (LPS) displayed by C3H/HeJ mice, namely endotoxemia and the intraperitoneal extravascular leukocyte responses to small doses of LPS, have been studied by Sultzer and found to be under polygenic control (1, 2). Recently, however, Watson and Riblet (3) presented evidence interpreted as indicating that a single gene was responsible for influencing both mitogenic (polyclonal) and immunogenic (specific) responses to LPS, by analysing crosses between LPS low-responder mice and high-responder strains. Since the B-cell unresponsiveness of C3H/HeJ mice has been shown to depend upon a pure defect in the subpopulation of B cells which interacts with and responds to LPS in the conventional strains,[1] the finding of Watson and Riblet is of great importance. Thus, it seems possible that the defective gene in C3H/HeJ mice would code for the B-cell surface structure involved in cell triggering, both in polyclonal (4) and in specific (3, 5, 6) antibody responses. According to our current view in B-cell activation, such "mitogen receptor" is the only structure on the B-cell surface which is competent to deliver activating signals to the resting cell (7). If this were the case, C3H/HeJ mice would be of great importance for elucidating the molecular mechanisms governing the generation and delivery of triggering signals. We report in this paper evidence on the inheritance of the genetical defect displayed by C3H/HeJ mice with regard to the direct B-cell responses to LPS as evidenced by polyclonal and specific responses as well.

Materials and Methods

C3H/HeJ (*H-2*^{k}) mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and bred in our colony for the last 3 yr. Other mice obtained from that source were also directly used in some experiments. The high-responder strains used in these experiments were C3H/Tif (*H-2*^{k}) obtained from Bomholtgaard, Rye, Denmark, and B10.5M (*H-2*^{b}) from our own colony.

LPS from *Escherichia coli* O55:B5 obtained by phenol-water extraction (8) was used throughout these experiments. The hapten (4-hydroxy-3,5-dinitrophenyl)acetyl (NNP) was conjugated to LPS as previously described (9) and the biological characterization of the conjugate used in the present

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experiments have been presented before (10). Culture and assay conditions for the study of polyclonal proliferative responses to LPS by mouse spleen cells, as well as for the induction of specific thymus-independent anti-NNP antibody responses by NNP-LPS were as described earlier (9, 11).

Results

C3H/HeJ LPS low-responder mice were crossed to mice of the LPS high-responder strain B10.5M. Proliferative responses to LPS in well defined serum-free culture conditions were studied in F_1 and F_2 hybrids and different backcrosses. A range of LPS concentrations were used in every experiment, since C3H/HeJ mice do mount low to intermediate responses to very high concentrations of LPS, which are paralytogenic for high-responder strains (reference 4 and footnote 1). The pattern of the dose-response curves is indeed the most clearcut difference between high and low responders.

In every instance, F_1 hybrids between those two strains of mice mounted proliferative responses to LPS which were in between the responses exhibited by each parent. This was always observed, irrespective of whether the F_1 hybrids were derived from a low- or a high-responder mother. F_2 hybrid mice clearly segregated in low, high, and intermediate responders. Backcrosses of F_1 hybrid mice to the low-responder parent, irrespective of the particular sex combination, segregated in intermediate and low responders, whereas backcrosses to the high-responder parent segregated in intermediate and high responders.

Results from these experiments are presented in Fig. 1 and Table I. Fig. 1 shows the results of a typical experiment. Controls of both high- and low-responder mice were always included and as can be seen B10.5M mice mounted significant responses to LPS concentrations as low as 0.1 μg/ml, whereas the low responder C3H/HeJ required 1,000-fold higher LPS concentrations (100 μg/ml) to

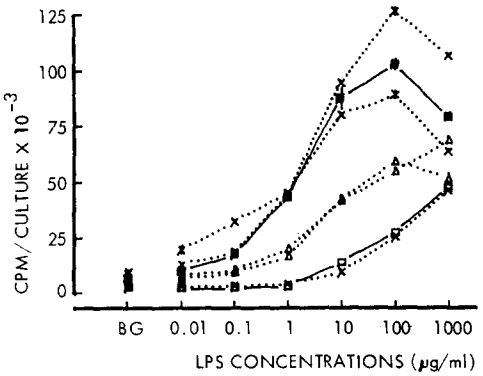

FIG. 1. Spleen cells from individual mice were stimulated by increasing concentrations of LPS for 2 days, in serum-free medium, in the microculture system (5×10^5 cells/culture, corresponding to a cell concentration of 2.5×10^6 cells/ml), and pulsed for the last 24 h of the culture period with tritiated thymidine. Results are shown as the mean ± 1 SE of the cpm obtained in triplicate cultures. B10.5M (■—■), C3H/HeJ (□—□), (C3H/HeJ × B10.5M) and (B10.5M × C3H/HeJ) F_1 hybrids (Δ···Δ), F_2 hybrids (×···×). As reported before (4), the responses to the PBAs, purified protein derivative from tuberculin, and dextran-sulfate, as well as to the T-cell mitogen con A were comparable in all mice.

TABLE I

Inheritance of B-cell Responsiveness to LPS by Crosses between C3H/HeJ and B10.5M or C3H/Tif Mice

Crosses	N*	Responsive pattern‡					
		High		Intermediate		Low	
		Observed	Expected§	Observed	Expected	Observed	Expected
		%	%	%	%	%	%
F_1 hybrids‖	20	0	0	100	100	0	0
F_2 hybrids‖	15	13	25	53	50	33	25
Backcrosses to high responder parent‖	12	50	50	50	50	0	0
Backcrosses to low responder parent‖	6	0	0	66	50	33	50
F_1 hybrids¶	2	0	0	100	100	0	0

* Number of identical crosses tested in each group.
‡ Results are given as percentage of the total number of individual mice tested in each group.
§ Expected percentages if a single, codominantly expressed autosomal gene determines responsiveness.
‖ Crosses between C3H/HeJ and B10.5M mice.
¶ Crosses between C3H/HeJ and C3H/Tif mice.

develop the same magnitude of response. It is also clear from the figure that both F_1 hybrids tested in this experiment show a dose-response curve which is intermediate to those of the parents, whereas the F_2 hybrids segregate into two high responders and one low responder. Differences in the magnitude of responses are much more clear at LPS concentrations from 1 to 10 μg/ml in our culture conditions, and with this LPS preparation. Table I shows the pooled results of many experiments totally involving 55 individual mice. In each case the responses were classified as low, intermediate, or high and the distribution of the different types of crosses by such categories is shown. A few mice which could not be safely ascribed to one of those classes were discharged from the table. Table I shows that F_1 hybrid mice resulting from crosses of low-responder C3H/HeJ and high-responder C3H/Tif, also behave as intermediate responders, suggesting that this pattern of inheritance is not restricted to the particular strain combination analysed above.

Watson and Riblet (3) have shown that polyclonal (nonspecific) unresponsiveness to LPS was linked in the backcrosses to the unresponsiveness to immunogenic (specific) challenge with LPS, thus providing strong support for the idea that both of these responses are controlled by the same triggering mechanisms (7). We also studied the responsiveness of different crosses to specific challenge with LPS, in the form of primary immune responses to NNP-LPS in vitro. As shown in Fig. 2, the specific responses to the hapten-LPS conjugate also follow the same pattern as above, namely they can be classified as high, low, and intermediate responses in the different individual mice. Most importantly, polyclonal and specific unresponsiveness segregate together in all instances. Since there is no indication of a variable region gene defect in the low responder mice (5), it can be assumed that Ig-mediated binding of NNP-LPS to the hapten-specific B cells is the same in low and high responder strains. Therefore, the magnitude of the responses most likely reflects direct B-cell responsiveness to membrane-bound concentrations of the mitogen (LPS), which is not different

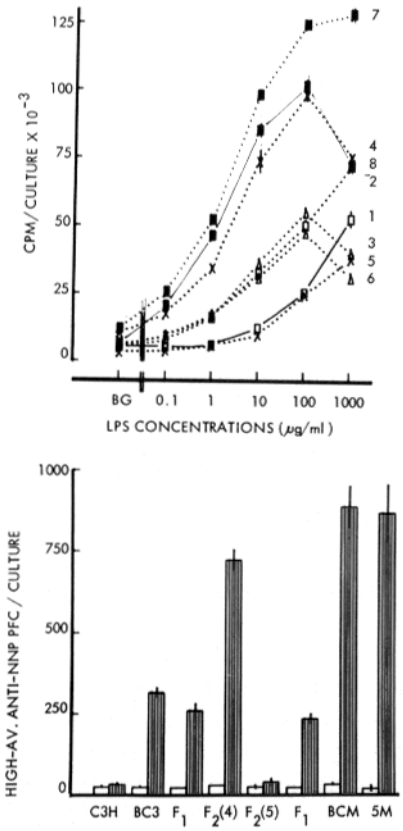

FIG. 2. In the upper part of the figure an experiment similar to that presented in Fig. 1 is shown. B10.5M (8) (■—■), C3H/HeJ (1) (□—□), F_1 hybrids (3 and 6) (△···△), F_2 hybrids (4 and 5) (×···×), backcross to B10.5M (7) (■···■), and backcross to C3H/HeJ (2) (□···□). The lower part of the figure shows the primary specific anti-NNP plaque-forming cell responses of spleen cells from the same individual mice to NNP-LPS. Anti-NNP responses were induced in serum-free medium (10^7 cells/ml/culture, in petri dishes), and assayed after 3 days of culture. Results are presented as the mean ± 1 SE of values obtained in triplicate cultures. Open bars, unstimulated cultures and hatched bars, NNP-LPS-stimulated cultures. From left to right, the various groups correspond to the numbers shown at the right-hand side in the upper part of the figure, and they are shown in parentheses for the F_2 hybrid responses.

between high and low responder cells, indicating fundamental differences in triggering receptors or mechanisms.

Discussion

The present results demonstrate that the B-cell unresponsiveness to LPS in C3H/HeJ mice is nearly absolute at polyclonal B-cell activator (PBA) concentrations below 100 μg/ml, but relative at higher LPS concentrations. It is unlikely that the responses observed to very high concentrations of LPS are due to contaminants in the endotoxin preparations other than LPS.[1] However, such a quantitative trait is easily interpreted in terms of the complete absence of the LPS-triggering receptor in these mice. Since C3H/HeJ B cells to have triggering

receptors/mechanisms for purified protein derivative from tuberculin and other PBAs (4), it is logical to assume that, in the absence of the "normal" LPS-triggering receptor, LPS can still interact and activate C3H/HeJ B cells via triggering receptors for other PBAs. The efficiency of this interaction with the "wrong" receptor is low and, therefore, activation requires very high LPS concentrations.

As pointed out above, the most clearcut difference between low and high responders is the pattern and the shape of the dose-response curve to LPS. The discrepancies between our results and those of Watson and Riblet (3) concern the responsiveness of F_1 hybrids between high and low responder mice. Differences could be due to different culture conditions, and different LPS preparations, but it is also possible that crosses between C3H/HeJ and B10.5M used in our experiments or between C3H/HeJ and C3H/Tif, are fundamentally different from F_1 hybrids between C3H/HeJ and CWB or DBA/2, as used in Watson's and Riblet's experiments. Another clear conclusion derived from the present experiments is that responsiveness to polyclonal-activating properties of LPS segregates together with specific responsiveness to antigen determinants on LPS in thymus-independent responses. As shown before, this is the case with LPS as well as with hapten-LPS conjugates, but only in situations where B-cell mitogenicity of the immunogen is critical for inducing a response (3, 5, 6). Thus, a thymus-dependent form of LPS is competent to induce a very good response in C3H/HeJ mice (5), since in this case, nonspecific triggering signals are provided by the accessory cell system (7).

The genetic mechanism responsible for the low response of C3H/HeJ mice cannot be clearly answered by these experiments. There are two main problems of interpretation. (*a*). The mechanism by which the F_1 hybrid shows an intermediate response and (*b*) the number of genes determining responsiveness.

(*a*) The most simple interpretation of the intermediate responsiveness of the F_1 hybrids is that high and low responsiveness are determined by codominantly expressed genes. The experimental findings were consistently that half or less than half of the cells responded in the F_1 hybrids, as compared to high responders, both in polyclonal and specific responses. The cellular mechanism leading to such a finding could theoretically be (i) only half the number of cells could be activated by LPS, the other half lacking the triggering receptors (a situation simular to allelic exclusion) and (ii) that all cells could be activated but at the doses tested only half did. If allelic exclusion is responsible for these findings the genes determining low responsiveness could be recessive or dominant. The latter possibility suggests codominant expression of the gene products, each F_1 hybrid cell having half the number of each gene product. However, in this situation it is to be expected that all cells should be activated by a higher concentration of the activator. The experimental findings did not substantiate this possibility, since the hybrids never reached the responder level even at high LPS doses.

(*b*) The second problem of interpretation concerns the number of genes involved. The results of the backcross and F_2 experiments shown in Table I are compatible with the existence of a single codominantly expressed autosomal gene determining responsiveness. This conclusion should be viewed with caution for a number of reasons, not the least important being the quantitative nature of the

difference between responder and low-responder mice and the arbitrary nature of the classification into three different response levels. However, alternative number of genes or different degrees of gene penetrance do not give results that fit the segregation data.

Summary

The inheritance of B-cell responsiveness to lipopolysaccharide (LPS) was studied in 55 crosses between mice of the low-responder strain C3H/HeJ and the high-responder strains B10.5M and C3H/Tif. F_1 hybrid mice between the low- and the high-responder strains, showed in every case responses which were intermediate between the responses obtained with each parent. The responsiveness among F_2 hybrid and backcross mice to either high- or low-responder parents, segregated into intermediate, high, or low categories, respectively. The present results are compatible with the hypothesis that responsiveness to LPS is determined by one single, codominantly expressed, autosomal gene. The capacity to develop a specific thymus-independent response to a hapten-LPS conjugate, also under genetical control, was found to segregate together with the capacity to develop polyclonal responses to LPS.

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