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Evolution of Cell Types and T-Cell Subsets in the Spleens of *Mycobacterium bovis* BCG-Resistant and *M. bovis* BCG-Susceptible Strains of Mice after Infection with *M. bovis* BCG

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In mice, the early host response to intravenous infection with small doses of dispersed *Mycobacterium bovis* BCG is controlled by the *Bcg* gene. After infection with a low dose of *M. bovis* BCG, Lyt-1$^+$ cells were generated in the spleens of BCG-susceptible mice (*Bcg*^{s}) in parallel with an increase in the proportion of phagocytic cells. Very few changes occurred in the splenic cell types of BCG-resistant mice (*Bcg*^{r}).

In mice, the early host response to intravenous infection with small doses of dispersed *Mycobacterium bovis* BCG is controlled by the *Bcg* gene (3, 5). BCG-resistant (*Bcg*^{r}) mice prevent net growth of *M. bovis* BCG in their reticuloendothelial tissues, whereas there is progressive multiplication of mycobacteria in the organs of BCG-susceptible mice (*Bcg*^{s}). The *Bcg* gene exerts its action at the early phase of the infection, before an immune response is elicited (5). Since the bacillary load which develops in mice infected with low doses of *M. bovis* BCG is controlled by the *Bcg* gene, this locus will have much influence on the quality and the quantity of the immune response in the infected mice during the later phase of the infection. In support of this concept, striking differences in the development of many parameters of immunological responsiveness, such as delayed-type hypersensitivity (DTH), formation of liver granulomas, splenomegaly, as well as resistance to a heterlogous pathogen have been detected between *Bcg*^{r} and *Bcg*^{s} mice after infection with small doses of *M. bovis* BCG (14).

In this report, the evolution of the cell types and T-cell subsets in the spleens of *Bcg*^{r} and *Bcg*^{s} mice was investigated. Two strains of mice typed as *Bcg*^{r} C3H/HeN and A/J and two strains typed as *Bcg*^{s} B10.A/SgSn and C57BL/6 were used. They were obtained from Charles River Breeding Laboratories, St-Constant, Canada or from Jackson Laboratory, Bar Harbor, Maine. Animals were infected intravenously via the caudal vein with a small dose (2.2×10^4 CFU) of *M. bovis* BCG strain Montreal (Institut Armand-Frappier, Laval, Canada). Spleens were harvested at various times after infection, and cell suspensions were prepared by standard procedures by using RPMI-1640 (GIBCO Laboratories, Grand Island, N.Y.) with 0.3% bovine serum albumin. A technique with acridine orange (J. T. Baker, Phillipsburg, N. J.) for the identification of phagocytic cells was used (6). B lymphocytes were enumerated by an immunofluorescence technique with fluorescein-labeled sheep $F(ab)'_2$ anti-mouse immunoglobulin (New England Nuclear Corp., Boston, Mass.) (8). Complement-mediated cytotoxicity was used to identify T lymphocytes. Briefly, single-cell suspensions were treated with a 1:40 dilution of anti-Thy 1.2 monoclonal antibodies (Cedarlane Laboratories, Hornby, Canada). Cells were incubated for 1 h at 4°C, washed, and incubated for 1 h at 37°C in the same volume of a 1:40 dilution of rabbit complement (low toxicity rabbit complement; Cedarlane Laboratories). Live and dead cells were scored by using trypan blue; Lyt phenotypes were enumerated by the sequential lysis method of Cantor and Boyse (1). Anti-Lyt 1.2, anti-Lyt 2.2, anti-Lyt 1.1, and anti-Lyt 2.1 monoclonal antibodies were obtained from Cedarlane Laboratories.

There were only slight differences in the relative proportion of the various cells in the spleens of C3H/HeN and A/J (both *Bcg*^{r}) mice at various times after BCG infection (Fig. 1). A slight increase in the proportion of phagocytic cells was observed 3 weeks after infection. In contrast, dramatic changes in the relative proportion of various cell types in the spleens of C57BL/6 and B10.A/SgSn (*Bcg*^{s}) mice occurred after infection. The relative proportion of T lymphocytes and B lymphocytes dropped during the course of infection, whereas the relative proportion of phagocytic cells steadily increased to reach approximately 33% at 3 weeks. The Lyt phenotypes of the spleen T lymphocytes of the various strains of mice after infection are shown in Table 1. There was a very significant ($P < 0.001$) increase in the percentage of Lyt-1$^+$ cells in the spleens of C57BL/6 and BIO.A mice starting 3 weeks after infection, such that at 4 and 6 weeks after infection, 79 to 86% of all T cells were Lyt 1$^+$. Concomitantly, there was a marked decrease of Lyt 123$^+$ cells. Such dramatic changes did not take place in the spleens of C3H/HeN and A/J mice (*Bcg*^{r}). There was an increase in the proportion of Lyt 1$^+$ cells, but this increase occurred much later and was less pronounced than that in the spleens of C57BL/6 and B10.A/SgSn strains.

As we have shown in a previous report, striking differences in the character and the magnitude of various indicators of cellular immunological responsiveness occur between *Bcg*^{r} and *Bcg*^{s} mice after infection with a low dose of *M. bovis* BCG (14). In parallel with the bacterial multiplication, we have demonstrated that susceptible mice develop a diffuse, severe granulomatous hepatitis which starts 2 weeks after the infection. The number of granulomas further increases to reach a peak at 3 weeks and remains elevated until week 7. Concomitantly, the splenic index increases dramatically and remains high until week 6. This increase in spleen weight is, at least in part, secondary to the presence of numerous granulomas. This study extends these earlier

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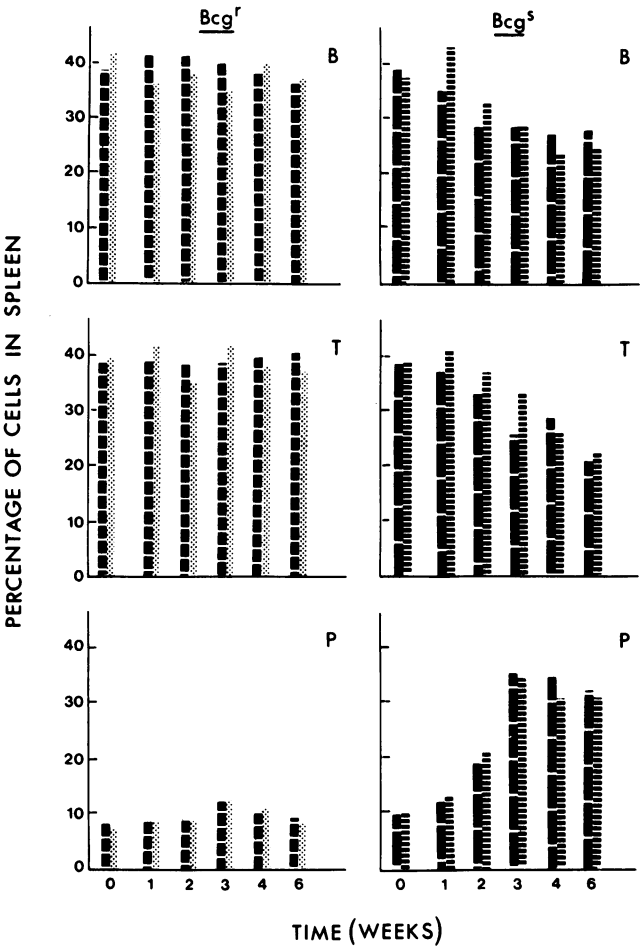

FIG. 1. Proportion of T lymphocytes (T), B lymphocytes (B) and phagocytic cells (P) in spleens of *Bcg*^{r} mice C3H/HeN (▩) and A/J (▤) and *Bcg*^{s} mice C57BL/6 (▤) and BIO.A/SgSn (▤) infected intravenously with 2.2×10^4 CFU of BCG Montreal. Each piece of data represents the arithmetic mean of 4 determinations. Standard errors of the mean ranged from 1 to 4%.

observations by showing considerable differences in the evolution of the splenic cell types and T-cell subsets between *Bcg*^{r} and *Bcg*^{s} mice during the course of infection with a low dose of BCG. The occurrence of major changes in the relative proportion of various cell types and T-cell subsets in *Bcg*^{s} mice occurred at the time of the downturn in the bacterial load and followed the same time course of other cellular immune parameters, mainly the development of splenomegaly, granuloma formation, and resistance to homologous (BCG) and heterologous (*Listeria monocytogenes*) pathogen (15).

In this study the Lyt phenotypes are defined at an operation level only. Functionally, some of the Lyt 2^+3^+ cells might be expressing the Lyt 1 antigen but at a density low enough to escape lysis by anti-Lyt 1 monoclonal antibodies plus complement. This is in light of the finding that Lyt 1 antigen is expressed on most T cells when the fluorescence-activated cell sorter technique is used (9). Caution is required when an association between a certain Lyt phenotype and a particular T-cell function is considered in view of the following observations. (i) Multiple in vivo interactions occur between the various subsets (4), and (ii) There is evidence which suggests that the Lyt phenotype shown by an active T cell may be altered during an actual immune response (13, 15). However, Lyt 1^+ cells have been shown to be involved in various immunological processes, help in the production of antibodies (1), production of lymphokines (2), delayed-type hypersensitivity (7, 12), as well as resistance in infection with parasites (10). Thus, the dramatic increase in the Lyt 1^+ that we observed in the spleens of infected *Bcg*^{s} mice, in parallel with the decrease of Lyt 123^+ cells may reflect the existence of a cellular immune response in these mice.

Thus, at the level of the commonly observed immune parameters (14) and at the cellular level as shown in this study, our model offers a promising probe in the dissection of host defense mechanisms in a series of step-wise processes. The *Bcg* gene acts early in the infection, before an immune response sets in. Obviously, distinct genetic systems controlling specific T-cell response to intracellular parasites will be elicited if the microbial burden persists. Nevertheless, the level of natural resistance will affect the

TABLE 1. Lyt phenotypes of splenic T lymphocytes of mice infected intravenously with a small dose of BCG[a]

Time after infection (wk)	Phenotype	Mouse strain							
		A/J		C3H/HeN		BIO.A		C57Bl/6	
		Control (%)	Infected (%)	Control (%)	Infected (%)	Control (%)	Infected (%)	Control (%)	Infected (%)
1	Lyt 1^+	41	28	39	43	42	46	36	37
	Lyt 23^+	17	11	21	16	16	12	17	20
	Lyt 123^+	32	55	38	30	31	35	50	33
2	Lyt 1^+	51	54	47	44	32	47	39	54
	Lyt 23^+	21	19	23	19	17	11	16	12
	Lyt 123^+	28	26	32	34	43	40	37	22
3	Lyt 1^+	43	40	37	46	34	68[b]	45	83[b]
	Lyt 23^+	18	31	15	17	11	14	21	6
	Lyt 123^+	26	26	37	36	42	11	36	7
4	Lyt 1^+	39	57[c]	43	59[c]	45	86[b]	49	84[b]
	Lyt 23^+	16	14	10	13	18	5	9	3
	Lyt 123^+	39	29	36	21	33	6	43	9
6	Lyt 1^+	47	59[c]	41	61[c]	47	80[b]	43	79[b]
	Lyt 23^+	22	17	17	10	16	8	16	11
	Lyt 123^+	31	19	29	19	26	9	33	9

[a] Each number represents the arithmetic mean of four determinations (Standard errors of the means varied from 1 to 4%).
[b] Statistically different ($P < 0.001$) from controls.
[c] Statistically different ($P < 0.01$) from controls.

quality and quantity of specific immune response. This is demonstrated clearly in our infectious model with *M. bovis* BCG Montreal, where the character and magnitude of the immune parameters studied can be strongly influenced by variations in bacterial load, resulting from the operation of a natural defense mechanism ascribed to the *Bcg* locus.

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