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Immunological Behavior After Mycobacterial Infection in Selected Lines of Mice with High or Low Antibody Responses

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Resistance and susceptibility to mycobacterial infection in the Biozzi high and low lines of mice which were genetically selected for their responses to heterologous erythrocytes have been found to be related to the innate ability of nonimmune macrophages to kill or inhibit the growth of the organisms during the first two weeks after infection and to their ability to mount specific and nonspecific immune responses. High antibody-producer mice were more capable of expressing cell-mediated immune parameters than low antibody-producer mice. A direct relationship was observed between the ability of bacteria (BCG vaccine) to multiply inside the reticuloendothelial system and the development of cell-mediated immunity, as measured by the delayed local reaction at the injection site, the lymphoproliferative response in the draining nodes, the tuberculin delayed-type hypersensitivity, the acquired resistance, and the adjuvant effect after BCG inoculation. In high line mice, apart from the inability of their macrophages to inhibit the early growth of bacteria, their lymphocytes in spleen and thymus were more capable of being stimulated in vitro by varying concentrations of living BCG. The data presented in this report are compatible with the hypothesis that a group of genes segregated in each line during the selective breeding controls the innate microbicidal activity.

Two lines of mice (high line [HL] and low line [LL]) have been genetically selected from Swiss albino stock for their respective high and low antibody formation after immunization with sheep erythrocytes (SRBC) (4). The extensive quantitative difference between them involves not only all classes of immunoglobulin but also antibody synthesis in response to a wide range of unrelated antigens (both thymus dependent and thymus independent) (7, 16). These humoral differences were shown to be related to the segregation of polygenic characters determined by a group of about 10 to 12 independent loci. In vivo and in vitro experiments have demonstrated that the functions of both B lymphocytes and macrophages were deeply affected by genetic selection (6, 38, 39). The HL and LL differ greatly in the cytodynamics of their B-cell responses (4) and in the macrophagic functions, namely those concerning the metabolism and presentation of the antigen to lymphocytes (39). By contrast, the T-cell-mediated immunity (CMI) exhibited by these strains is quantitatively similar with regard to graft-versus-host (9) and allograft reactions (24), phytohemagglutinin responsiveness (25), and picryl-chloride sensitization (34).

Their natural or acquired resistance to experimental tumors (5) and infections has been the subject of several reports (2, 10, 12, 18). These selected lines are thus considered as useful tools for the in vivo study of immune-related phenomena and relative roles of the humoral and cellular components of the immune response to pathogens.

Natural and specific acquired resistance to facultative intracellular parasites such as *Salmonella*, *Brucella*, and *Yersinia* has been shown to be higher in the LL than in HL (4, 10, 12), which seems to be the result of the participation of macrophages in the resistance of the host. But in these different experimental systems, one cannot rule out the direct or indirect participation of a particular subclass of antibodies which can actively participate with macrophages (37). With other obligatory intracellular parasites such as *Listeria monocytogenes* or *Mycobacterium* sp., it has been shown that immune serum does not participate in acquired resistance and also that CMI can be induced only with living bacteria, which multiply inside phagocytic cells (29). Thus, it was of interest to study some immunological parameters in these selected lines of mice after a mycobacterial infection.

MATERIALS AND METHODS

Mice. HL and LL antibody producers were raised from breeders kindly provided by G. Biozzi (Fondation

Curie, Paris, France), in a protected environment in our animal facilities. Breeders and progeny were fed with a sterilized, vitamin-complemented diet, and sterile water (pH 3) was given ad libitum. Most experiments used female mice from selection 1 (as described in reference 7), 6 weeks old at the start of each experiment. Some male mice were also used and are indicated in the text.

Microorganism. *Mycobacterium tuberculosis* var. *hominis* strain H37 Rv was obtained from the Pasteur Institute mycobacterial culture collection and was harvested on day 7 of culture in Sauton medium in the tuberculosis unit (H. David, Institut Pasteur). Mice were injected in the lateral tail vein with 0.2 ml of sterile saline (0.8% sodium chloride solution) containing 1.6×10^6 viable H37 Rv. Survival times were recorded three times per week thereafter.

M. tuberculosis var. *bovis* strain BCG was obtained in deep culture as previously described (19). Mice were injected either intravenously (i.v.) in the lateral tail vein with a volume of 0.2 ml of sterile saline containing an appropriate amount of viable BCG or subcutaneously (s.c.) in the left hind footpad (LHFP) with a volume of 0.04 ml of saline containing an appropriate dilution of viable BCG. Counts of viable BCG were performed at varying time intervals in spleen, liver, lungs, or popliteal draining nodes after i.v. or s.c. challenge, respectively, by plating dilutions of homogenized organs on Middlebrook 7H10 medium. These counts were expressed to the $\log_{10}$, and the geometric mean per group was calculated.

L. monocytogenes was kindly donated by R. M. Fauve (Institut Pasteur). Bacteria were cultivated in Trypticase soy broth for 6 h and then diluted in saline to an appropriate dilution, which corresponded to 0.2 50% lethal dose. Mice were inoculated i.v. with 0.5 ml, and growth curves of *L. monocytogenes* in spleen and liver were made by counting of bacteria-forming colonies after plating dilutions of homogenized spleen and liver as previously described (28).

Antigens. SRBC in Alsever solution were obtained weekly from the same animal. They were stored at 4°C and washed three times before being resuspended to the desired concentration in normal saline.

Lyophilized purified protein derivative (reference 79610, Institut Pasteur Production), kindly donated by J. Augier (Institut Pasteur), was diluted in sterile saline to a concentration of 5,000 tuberculin units and stored at −20°C in small vials.

DLR. For testing of delayed local reaction (DLR), mice were injected with 2×10^6 BCG in the LHFP in a volume of 0.04 ml. Regularly thereafter, the footpad swelling was measured with a dial caliper reading to 0.05 mm. Reactions were expressed as the difference in thickness between feet that had received the BCG injection and those that had not, as previously described (19).

Measurement of cellular responses in popliteal nodes. The extent of cell proliferation in response to BCG was measured in popliteal lymph nodes of mice inoculated in the footpad as previously described (30). To measure and express the relative rates of cell division in responding nodes, [^{125}I]uridine incorporation into deoxyribonucleic acid was examined as reported elsewhere (20). In brief, at intervals after immunization, five mice from each group received an i.v. injection of 0.5 μCi of [^{125}I]uridine (90 to 110 μCi/μg) obtained from the Radiochemical Centre, Amersham, United Kingdom. Two hours later, the popliteal nodes were excised, placed in individual plastic tubes, and counted in a gamma spectrometer for 10 min, with [^{125}I]uridine uptake expressed as counts per minute. The results are expressed as the percentage of the relative counts per minute per node to the relative counts per minute of the inoculum (0.5 μCi/mouse): [(counts per minute/node − counts per minute/background)/(counts per minute/inoculum − counts per minute/background)] × 100.

DTH. The delayed-type hypersensitivity (DTH) reaction was measured as described elsewhere (21). In brief, variations of the footpad thickness were measured after injection of 0.04 ml of saline containing the eliciting antigen into the right hind footpad (RHFP). Reactions were expressed as the difference in thickness between feet that had received injection of eliciting antigen and those that had not.

Antibody titration. Hemagglutinin titers were determined in micotitration trays as described previously (31). In brief, a 0.1-ml volume of heat-inactivated serum was diluted serially in isotonic Veronal buffer, to which was added an equal volume of 1% packed SRBC. After 30 min at 37°C, the trays stood overnight at 4°C. Mercaptoethanol-resistant titers were determined in the same way with serum which had been reduced for 0.5 h at room temperature with mercaptoethanol at a final concentration of 0.1 M.

In vitro lymphoproliferative index. Spleens and thymuses were aseptically removed from mice of the two lines. Cells were washed twice in RPMI 1640 containing 10% fetal calf serum and enumerated in a hemocytometer. Viability, as assessed by dye exclusion with trypan blue, was not less than 90%.

A total of 3×10^5 nucleated cells in 50 μl of 10% fetal calf serum–RPMI 1640 were cultured in microculture plates for 24 h with 100 μl of varying numbers of live BCG. Then 50 μl of RPMI containing 1 μCi of tritiated thymidine ([^{3}H]thymidine) was added to each well 18 h before harvest. The cells were harvested onto glass fiber filter papers, and the incorporation of [^{3}H]thymidine into deoxyribonucleic acid was determined in a liquid scintillation spectrometer and expressed as counts per minute; then the stimulation index, i.e., the ratio of BCG-stimulated cells to background, was calculated.

RESULTS

Criteria of comparison of Biozzi HL and LL mice. Before undertaking the comparative study of a mycobacterial infection of these lines of mice, a preliminary experiment was done to verify that the offspring of the two lines retained the characteristics of HL or LL. The difference in antibody production between the two lines was perfectly conserved by the offspring.

The underlying mechanism of this difference has been described as the greater capacity of phagocytic cells of LL to destroy antigen. This was also confirmed by using *L. monocytogenes*,

a bacteria which multiplies intracellularly in macrophages in vivo. Thus, the offspring of the two lines was tested with an i.v. injection of 2.4×10^5 *L. monocytogenes*. The spleens and livers of five mice per group were removed 6, 24, 48 and 72 h afterwards, and the number of bacteria forming colonies was counted. The results of such an experiment are given in Fig. 1 and confirm the preliminary results reported by Fauve (cited in reference 2). Note that at 48 and 72 h, a statistically significant difference was observed ($P < 0.005$) only in livers and not in spleens. Therefore, the offspring of HL and LL reacted identically to their parents in regard to antigen processing, and it was these offspring which were used to study different parameters of the immune response after mycobacterial infection.

In a previous report (30), it was shown that an injection of live BCG in mice caused alterations of the immune response, which when evaluated permitted the quantitation of the virulence of a particular strain of BCG. But with the same strain of BCG cultivated and stored under the same conditions, the measure of these immune parameters after BCG infection would permit the comparison of genetically different hosts. Thus, the different criteria of evaluation (22) were used with HL and LL mice.

Lymphoproliferative response and BCG growth in draining node. After an s.c. injection of BCG, proliferation of lymphoid cells in the paracortical area can be observed, and this proliferation varies proportionally with the dose and time after infection (30). Two groups of female HL and LL were injected with 4.8×10^6 live BCG in the LHFP and were sacrificed at different times thereafter. The results are shown in Fig. 2. Note a statistically significant difference ($P < 0.001$) starting at day 10. On days 3, 6, 10, and 14, five mice from each group were also sacrificed, and the number of BCG colony-forming units in popliteal draining nodes was determined. As shown in Fig. 3, there was less multiplication of BCG in the LL mice than in the HL mice. In conjunction with the ability of BCG to multiply locally and in the draining node, it was recently shown that the magnitude of the DLR in mice is one of the criteria for evaluating specific and nonspecific immune responses after a local injection of BCG vaccine (20). The following experiment measured DLR in mice of HL or LL after an s.c. injection of BCG.

DLR after BCG infection. Ten female HL and LL mice were injected with a single dose of 2×10^6 live BCG into the LHFP, and the local reaction was measured regularly thereafter. No difference was observed in the 24-h reaction, but

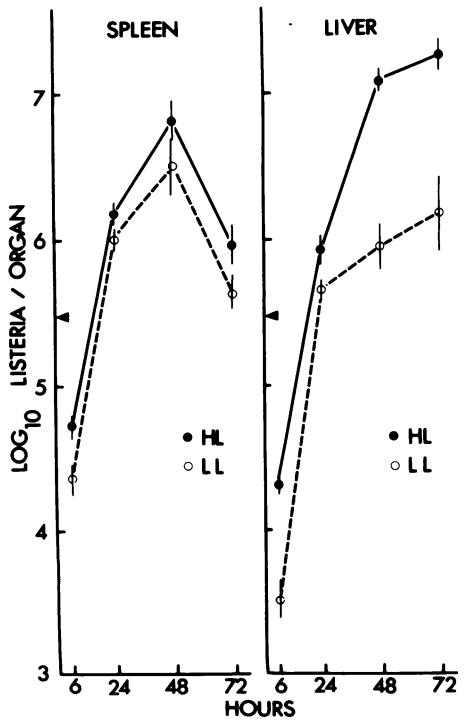

FIG. 1. *Growth curve of L. monocytogenes in spleen and liver of HL and LL mice injected i.v. with 2.4×10^5 viable organisms (arrow). Means of five mice ± standard error of the mean.*

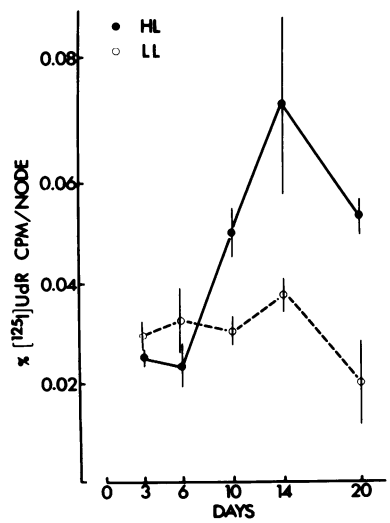

FIG. 2. *Dynamics of the percentage of [^{125}I]uridine ([^{125}I]UdR) incorporation into deoxyribonucleic acid in popliteal draining lymph nodes responding to 4.8 $\times 10^6$ viable BCG in HL and LL mice. Means of five mice ± standard error of the mean.*

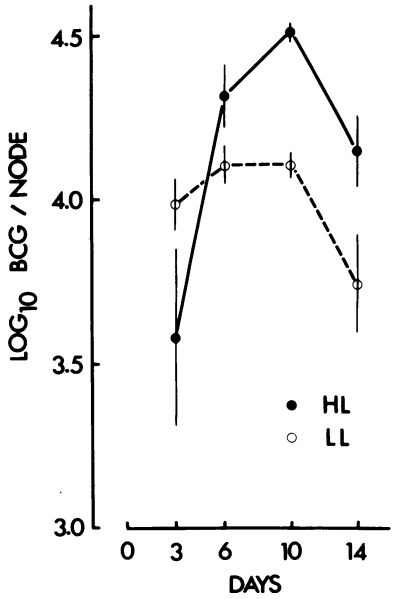

FIG. 3. *Growth curve of viable BCG recovered from popliteal draining lymph nodes after an injection in the LHFP of 4.8 × 10⁶ viable BCG in HL and LL mice. Means of five mice ± standard error of the mean.*

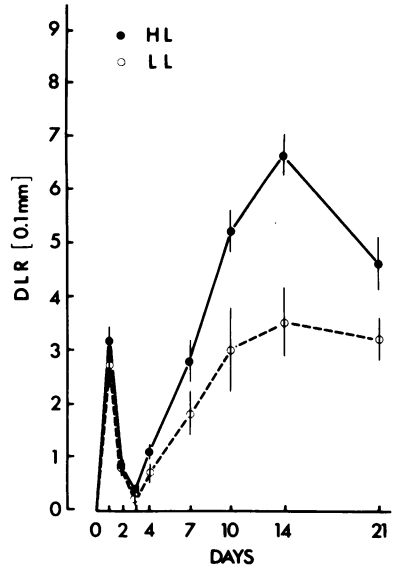

FIG. 4. *Development and decay of the DLR after an injection in the LHFP of 2 × 10⁶ viable BCG in HL and LL mice. Means of five mice ± standard error of the mean.*

as shown in Fig. 4, a statistically significant difference appeared starting on day 10 and continuing until day 21 ($P < 0.01$). However, this reaction also measured the formation of a granuloma which was not necessarily T dependent. It would have increased in size whether or not a specific reaction was present, but when a specific T-cell component exists, this DLR is always of a higher magnitude (19). Thus, this DLR measures simultaneously the granulomatous capacity of a particular strain of BCG and also the individual's own capacity to mount a specific immune response to BCG, which is a CMI response (20). The specific immune response is usually observed in vivo by measuring the cutaneous reaction with a non-granulomatous reagent such as tuberculo-protein. This was done in the following experiment.

DTH to tuberculin after BCG infection. HL and LL female mice were inoculated with 2 × 10^6 live BCG in the LHFP and on day 21 were tested with an injection of 200 tuberculin units in the contralateral footpad. The footpad swelling was measured during the hours which followed the eliciting injection. Again, there was a significant interline difference ($P < 0.001$), as shown in Fig. 5. When the same experiment was repeated comparing the tuberculin sensitivity in males and females of the two selected lines, there was a more marked difference between the

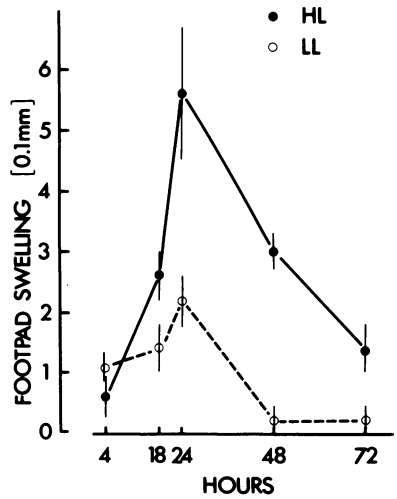

FIG. 5. *Time course of the increase of the RHFP thickness to an eliciting dose of 200 U of tuberculin in HL and LL mice sensitized 21 days previously with 2 × 10⁶ viable BCG given into the LHFP. Means of five mice ± standard error of the mean.*

females of the two lines than between male HL and LL mice. When DLR was measured, the interline difference was still present in female mice, but was not significant in males of these two lines on day 21 (Table 1). These results indicate some hormonal control of the immune response to BCG.

The specific immune response of mice to BCG

TABLE 1. *DLR and DTH measured on groups of female and male HL and LL mice*[a]

Line	DLR[b]		DTH[c]	
	♀[d]	♂[e]	♀[d]	♂[d]
HL	4.89 ± 0.51	3.78 ± 0.64	5.60 ± 1.02	4.00 ± 0.91
LL	2.90 ± 0.54	3.70 ± 0.60	2.20 ± 0.37	2.60 ± 0.40

[a] Measurements were taken 21 days after sensitization with 2×10^6 viable BCG given in the LHFP.

[b] The DLR was measured at the injected site, and results are expressed on the difference of thickness between feet that had been injected (LHFP) and feet that had not been injected (RHFP). Means of five mice ± standard error of the mean.

[c] The DTH represents the 24-h increased footpad thickness after an eliciting injection of 200 tuberculin units in the RHFP. Means of five mice ± standard error of the mean.

[d] $P < 0.01$.

[e] Not significant.

infection is always associated with a nonspecific immunostimulation which can be measured by several criteria: increase in colloidal carbon clearance (3) and nonspecific bacterial resistance (5, 35). One other criterion for measuring this immunostimulation was recently described; it consists of evaluating the adjuvanticity of BCG for DTH to heterologous erythrocytes in mice (32). The following experiment was performed to evaluate the adjuvanticity of the same strain of BCG in the two lines of mice.

Adjuvant action of BCG. Two groups of HL and LL female mice were injected i.v. with 5×10^8 SRBC. Six days later, they received an eliciting dose of 10^8 SRBC in the LHFP, and DTH reaction was measured 24 h later. As indicated in Fig. 6, no DTH was detected, as previously described when a supraoptimal dose of antigen was used (21). On the other hand, when mice were pretreated with an i.v. injection of 1.1×10^7 live BCG 2 weeks before injection of 10^8 SRBC, DTH measured in the LHFP was much greater in the HL mice (Fig. 6). The difference between the DTH levels in HL and LL was statistically significant ($P < 0.001$). The different groups of mice were bled on day 7 post-immunization, and the circulating specific antibody levels were determined. The total amount of antibodies was lower in BCG-pretreated LL than in non-BCG-pretreated HL. This difference in level of immunostimulation induced with the same dose of BCG could be explained by a difference in BCG-specific immune response which is either due to the absence of, or a lower level of, BCG multiplication in the LL mice. This was tested in the following experiment.

Growth of BCG in different organs after i.v. injection. Two groups of male HL and LL mice were injected i.v. with 3.0×10^6 live BCG, and viable counts were made for spleen, liver, and lungs on days 2, 21, and 35. The results in

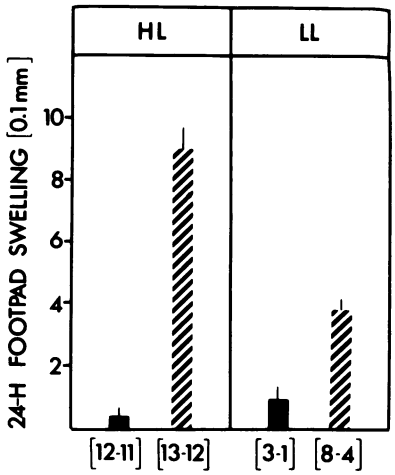

FIG. 6. *Effects of BCG treatment on the levels of 24-h DTH reaction to an eliciting dose of 10^8 SRBC in the LHFP on day 6 in response to an i.v. injection of 5×10^8 SRBC in HL and LL mice. A dose of 1.1×10^7 viable BCG was injected i.v. 14 days before immunization. Means of five mice ± standard error of the mean. The $\log_2$ hemagglutin titer (total/mercaptoethanol resistant) found on day 7 in pooled serum obtained from the preceding groups of mice is given below the graph in brackets. Symbols: ▨, BCG; ■, no BCG.*

Table 2 show that on days 2 and 21 more viable BCG were found in spleens and livers of HL mice, but on day 35 there were more bacteria in the lungs and livers of LL mice. This seems to indicate a lack of specific immune response in these LL mice, the bacteria being able to persist, which was associated with the absence of detectable immune response at the periphery (Fig. 5).

Since BCG is not lethal in mice, the H37Rv strain of *M. tuberculosis* was chosen for use in the following experiment which measured natural and acquired resistance of the HL and LL mice.

Natural and acquired resistance to *M. tuberculosis* H37Rv. Groups of female HL and LL mice were pretreated with 2.0×10^6 live BCG given s.c. in the LHFP and 21 days later, with two other groups of control female HL and LL, were challenged i.v. with H37Rv. As can be seen in Fig. 7, there is a significant difference in natural resistance between HL and LL mice, the LL dying much sooner than the HL (average survival time: HL, 4.5 months; LL, 2.5 months). In BCG-immune mice, at 6.5 months, all the LL mice are dead, whereas 40% of HL mice are still alive. Resistance represents a synergistic effect of the action of macrophages and thymus-dependent committed cells, but the T-cell induc-

TABLE 2. *Viable bacillary counts[a] recovered from spleen, liver, and lungs at varying times after an i.v. injection of 3 × 10⁶ viable BCG in HL and LL mice[b]*

Line	BCG count in spleen			BCG count in liver			BCG count in lungs		
	Day 2	Day 21	Day 35	Day 2	Day 21	Day 35	Day 2	Day 21	Day 35
HL	5.58 ± 0.21	5.09 ± 0.16	4.17 ± 0.25	6.52 ± 0.16	5.54 ± 0.15	3.95 ± 0.55	4.04 ± 0.08	4.51 ± 0.31	≤2.95
LL	5.16 ± 0.29	≤3.95	4.03 ± 0.28	6.12 ± 0.21	4.77 ± 0.34	4.35 ± 0.47	4.00 ± 0.06	4.41 ± 0.25	4.22 ± 0.30

[a] Log_{10} viable counts were performed by serial dilutions and surface plating in duplicate on Middlebrook 7H10 medium. Geometric means ± standard error of the mean.

[b] Groups of five male mice.

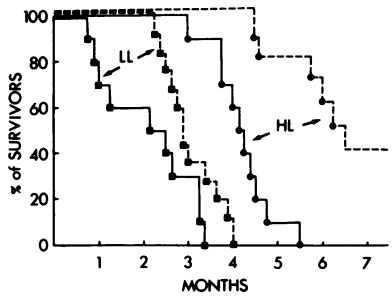

FIG. 7. *Effects of BCG treatment (dotted line) on the percentage of survivors after an i.v. injection of 1.6 × 10⁶ M. tuberculosis strain H37Rv in HL and LL mice. A single dose of 2 × 10⁶ viable BCG was given s.c. into the LHFP 21 days before the challenge. Control LL (■——■) and BCG-pretreated LL (■- - -■) mice died sooner than the control HL (●——●) mice, and after month 6, 50% of the BCG-pretreated HL (●- - -●) mice were still alive. Groups of 10 to 12 mice.*

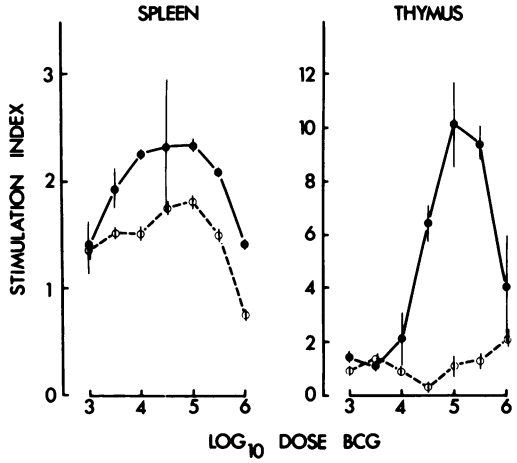

FIG. 8. *Stimulation index (SI) from spleen and thymus nuclear cells cultivated in vitro in 10% fetal calf serum–RPMI 1640 medium containing varying numbers of viable BCG. Triplicate of 3 × 10⁵ nucleated cells from lymphoid organs of HL (●) and LL (○) mice were incubated in a humidified, CO_2-enriched atmosphere, at 37°C for 48 h. Means of three mice ± standard error of the mean.*

tion in vivo seems to depend directly on the capacity of BCG to multiply inside the phagocytic cells. Thus, it was of interest to measure the stimulating activity of BCG on lymphocytes in short-term culture. This was tested in the following experiment.

Stimulating effect of live BCG on spleen and thymus cells. Since the backgrounds for the two cell lines were not identical, the results were expressed in terms of stimulation index. The HL cell background of [^{3}H]thymidine incorporation into deoxyribonucleic acid after 48 h of culture in normal medium was always higher than that of LL, as reported elsewhere (38). The spleen size and number of nucleated viable cells per spleen were greater for HL than for LL mice, as was the case also for the thymus (data not shown). As shown in Fig. 8, BCG leads to a much larger stimulation index for HL than LL cells, with the difference especially marked between the thymus cells of the two lines.

DISCUSSION

Overall results in this report demonstrate a better specific and nonspecific immune response in HL than in LL mice (selected for antibody production) in regard to a mycobacterial infection. Since specific antibodies are not involved in cell-mediated acquired resistance to intracellular parasites such as BCG, some other mechanism(s) must be responsible for the interline difference. Antimycobacterial acquired immunity is the result of cell cooperation between macrophages and T-committed lymphocytes (23). Our results indicate that CMI is better developed in HL than in LL mice. However, these results seem to be in direct contradiction to other published results which did not describe any T-cell-mediated interline difference (9, 10, 24, 34). But this might only be an apparent contradiction since the absence of interline difference was observed with nonreplicating antigens, such as those involved in skin graft rejection, and contact-sensitizing antigens. The absence of a difference between the two lines regarding CMI responses to nonreplicating antigen

could be caused by two related phenomena: on one hand, the observed equal capacity of lymphocytes to be stimulated by specific antigens or by mitogens as was previously described with phytohemagglutinin (25); on the other hand, according to the results presented by Howard et al. (15), the macrophage metabolic pathway of antigen-induced antibody synthesis could be different from that inducing CMI (4). However, for intracellular parasites which multiply in vivo only in phagocytic mononuclear cells, the intervention of macrophages appears to predominate in the induction of specific CMI (29). When nonliving, heat-killed *M. bovis* strain BCG were injected in Swiss mice, no evidence of acquired resistance and no sign of alteration of the immune parameters of the host were detected (30). It has been shown also that certain phenotypes, such as a streptomycin-resistant mutant which cannot grow in normal mice (11), do not provoke a cellular response in the regional lymph node (30). In the present report, macrophages of LL mice, which have been described to have an increased lysosomal enzyme content (39) and a greater ability to kill bacteriophage T4 more rapidly (16), can inhibit the multiplication of *L. monocytogenes* more readily (Fig. 2) and were also shown during the early stage of the infection to inhibit multiplication of BCG in vivo. Since intramacrophagic multiplication of the bacteria seems to be prerequisite for the production of acquired resistance in LL mice, only a very small specific immune response was induced, as demonstrated by various parameters: lymphoproliferation in draining nodes, tuberculin sensitization, DLR, and adjuvant activity.

There are several hypotheses for the requirement of live bacteria for the production of CMI. One could be the presence of replicating antigens, in adequate quantities, which are produced while the bacteria are multiplying and which do not exist or are masked when dead bacteria or their extracts are used. On the other hand, the production of mediators during the course of intramacrophage mycobacterial multiplication might cause the differentiation and selection of a particular subpopulation of lymphoid cells in lymphoid organs (1). These two phenomena could be associated, as indicated by the results of Mackaness and co-workers (33). High levels of T-cell activity were obtained only when T-cell-potentiating procedures (i.e., BCG) were used to immunize mice with soluble semipurified culture filtrate antigens from *L. monocytogenes*. They were manifested both as DTH in immunized donors and by transfer of protective immunity to *L. monocytogenes* in unimmunized recipients. These results indicate the necessary association of the two phenomena, one inducing the selectivity of particular subsets of lymphocytes involved in CMI and a second one participating in the specificity of these committed lymphocytes.

Stimulation in vitro of spleen cells, but especially thymus cells, of HL with BCG (Fig. 8) seems to indicate a preferential ability of these mice to respond to mycobacterial antigens. However, this marked interline difference, studied in whole-cell populations, should be analyzed in regard to the different populations of responsible cells. Adherent cells could exert an effect by producing one or more mediators, such as lymphocyte activating factor, which could be one explanation for the interline difference. This approach is currently being tested in our laboratory. Another possibility is that apart from the quantitative effect of mycobacterial multiplication in macrophages, better antigen presentation mediated by macrophages from the HL mice could provoke a higher specific response of T cells. Preliminary results in this laboratory have shown a marked interline difference (the HL mice gave a higher tuberculin response), after s.c. immunization with killed BCG injected with Freund incomplete adjuvant, for tuberculin hypersensitivity (Lagrange, unpublished data).

But another point needs to be taken into account. As reported by Youmans and Youmans (41), the increased susceptibility of a particular mouse strain, as evaluated by mean survival time, was due to their greater DTH to mycobacterial antigens (40). In a series of preliminary experiments, these results were reproduced in this laboratory, in particular with C57Bl/6 and C3H/HeN mice. The latter developed only a weak DTH to tuberculin, whereas the former presented strong reactions after BCG infection. According to the literature, these mice were more susceptible and died sooner after a challenge with H37Rv (13, 36) or after a tuberculin injection (14). But here, with the Biozzi mice, the normal or BCG-vaccinated LL mice died earlier than the normal HL mice after a challenge with H37Rv (Fig. 7). And these LL mice presented only weak reactions to tuberculin after BCG inoculation. Thus, the physiopathological pathways of death after mycobacterial infection must differ in susceptible Biozzi LL and C57Bl/6 mice.

After BCG vaccination (Fig. 6), in the same line, a sexual difference has been noticed. This difference observed among males and females has already been described for tuberculosis, females being more susceptible during their reproductive years (17). Lurie (26) and others have studied the effects of administration of various

hormones on resistance to experimental tuberculosis, and estrogen was shown to augment the natural capacity of nonimmune macrophages to kill bacteria (27). Thus, when the response to mycobacteria in males or in females of the LL mice was compared, females gave a lower response because of their higher natural antibacterial macrophage activities.

Data presented in this report confirm the necessity of bacterial multiplication as a prerequisite for the production of specific DTH and acquired resistance against intracellular microorganisms. Thus, with higher levels of natural immunity as manifested by higher macrophage antimicrobial capacity, ordinary artificial immunization with living bacterial vaccine might be ineffective.

From the observed results with the Biozzi mice selected for their antibody-producing ability, the explanation of certain facts observed after BCG vaccination in humans could be made. In particular, the fact that negative tuberculin reaction after repeated injections of BCG vaccine was observed could be explained by the ability of macrophages from such individuals to inhibit the intracellular multiplication of BCG and thus prevent the production of specific immunity. Experiments are currently being undertaken in this laboratory to observe the reactivity of the LL mice after multiple injections of BCG or after varying doses of vaccine to modify the tuberculin reactivity of these mice. Following the same line of reasoning, evaluation of the antibody levels produced by vaccines such as tetanus toxoid or diphtheria toxoid in tuberculin-negative individuals after BCG vaccination could permit the resolution of a part of this problem. However, other mechanisms could also be responsible for the negativity of the skin test after a BCG vaccination, such as the existence of cells inhibiting lymphocyte stimulation (8) or a previous sensitization with another strain of mycobacterium species (Lagrange, manuscript in preparation).

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