

Influence of Route of Inoculation on Anti-*Mycobacterium lepraemurium* Antibody Isotypes in Murine Leprosy¹

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Resistance to mycobacterial infection, both in man and mice, is mainly dependent on the development of cell-mediated immunity, without involvement of protective antibodies (8, 17, 22). However, anti-mycobacterial antibodies to genus-specific or species-specific epitopes are produced, and have been reported to vary across the clinical spectrum, and seem to correlate to the bacterial load (1, 3, 15). More recently, the discovery of a characteristic phenolic glycolipid-I (PGL-I) from *Mycobacterium leprae* has opened new perspectives in the field of serodiagnosis of the subclinical stages of leprosy infection (4, 5, 12, 23). However, serial individual antibody assessments are needed to ascertain whether antibody is a reliable indicator of infection and to predict accurately those among household contacts with negative cellular immunity tests who will develop clinical leprosy, particularly the multibacillary form of the disease.

In this regard, experimental mycobacterial infection models could be very useful in studying the sequence of appearance of anti-mycobacterial antigen antibodies in relation to the ability to resist the infection. The use of antibody assays in mice experimentally infected with *M. lepraemurium* (MLM), which induces a progressive chronic infection mimicking subpolar forms of human leprosy, could provide valuable information about early antibody production and its predictive value for the development of T-cell-dependent protection. Previous studies have indicated that the magnitude of anti-MLM antibody response correlated with the bacterial load, and showed that the

amount, the predominant isotype, and the species specificity of the anti-MLM antibodies produced during MLM infection were also influenced by genetic factors independent of those controlling the resistance to the infection (9, 11).

In the present work, anti-MLM IgG and IgM antibody production was studied in a single mouse strain, C57BL/6, using different doses and routes of inoculation to obtain different patterns of MLM growth and dissemination. Indeed, the resistance of MLM infection of the C57BL/6 mouse strain has been shown to be greatly influenced by the route of inoculation (6, 10, 13, 20). When C57BL/6 mice were infected intravenously (i.v.), they showed bacillary growth and dissemination; whereas when infected subcutaneously (s.c.), they showed a control of local bacillary growth and dissemination of bacilli. A high and predominant anti-MLM IgM antibody response was observed in heavy disseminated infection in mice inoculated i.v.; whereas IgG antibodies were produced at a higher level than IgM in the controlled infection seen in mice inoculated s.c. Thus, isotype analysis of anti-mycobacterial antibodies might represent a valuable tool to evaluate the individual's resistance to mycobacterial infections.

MATERIALS AND METHODS

Mice. Female, 8-week-old, C57BL/6 inbred mice were obtained from the Pasteur Institute (Paris, France).

MLM infection of mice. Mice were inoculated intravenously (i.v.) or subcutaneously (s.c.) with 10^5 or 10^8 MLM. The strain of MLM kindly donated by P. H. Lagrange was maintained as previously described (10).

MLM counting in infected mouse spleens and foot pads. At different times after the infection, the number of acid-fast bacilli (AFB) were enumerated in the infected or-

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gans using the auramin staining method as already reported (¹⁰).

Preparation of MLM sonicate. Heat-killed MLM (1 hr at 60°C) were sonicated, centrifuged at $100,000 \times g \times 60$ min, aliquoted, and frozen at -20°C until used, as previously described (⁹).

Anti-MLM sonicate total Ig, IgM, and IgG antibody measurement. At different times after the infection, the infected mice were bled by retro-orbital puncture. The sera were separated and stored at -20°C until used. Sera from age-matched uninfected mice were collected and used as normal controls. Circulating total Ig antibodies to MLM sonicate were assayed using an ELISA as already reported (⁹). Then for each serum IgM and IgG antibodies to MLM sonicate were concurrently measured using the same method. MLM sonicate was coated at a concentration of 5 µg protein/ml into microtiter plates (Immunlon 2; Dynatech, Springfield, Virginia, U.S.A.) and dried at 37°C overnight. Plates were then saturated with phosphate-buffered saline (PBS) containing 5% bovine serum albumin (PBS-BSA) for 1 hr at room temperature. Thereafter, twofold serial dilutions of each serum (from 1/20 to 1/640) were distributed into plates and left for 2 hr at 37°C. The serum dilutions used to assess the antibody activity varied with time of infection, but were constant at each time assessed. Plates not coated with MLM were also set up and served as controls for background measurement. The plates were then washed with PBS, and peroxidase conjugated goat anti-mouse Ig, µ, or γ-chain antibodies (Biosys, Compiègne, France) were added to each well at a final concentration of 1 µg/ml in PBS-BSA. The plates were incubated in the dark for 2 hr at 37°C. After washing, 100 µl of a solution of 0.4 mg/ml orthophenyl ethylenediamine (OPD; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) in citrate phosphate buffer (0.15 M, pH 5) containing 0.04% H₂O₂ was distributed into each well and incubated for 30 min at 37°C. The reaction was stopped by adding 50 µl of 2.5 N H₂SO₄. The optical densities (OD) were read at 492 nm.

Sera from uninfected animals served as normal controls. A slight nonspecific binding activity was observed in 9 out of 41 normal sera when total Ig anti-MLM anti-

body activity was assessed. For anti-MLM IgM and IgG antibody activities, a pool of the 41 uninfected sera was used as a negative control. No IgM activity was ever found in the pooled uninfected sera. A slight non-specific IgG binding was sometimes observed, in which case it was subtracted individually from the results obtained with infected sera. For each infected or uninfected serum, ΔOD was calculated by subtracting the OD without antigen from the OD obtained with MLM sonicate. ΔOD was plotted against the log of the reciprocal dilution of each serum.

In each plate, a reference serum obtained from highly infected C57BL/6 mice was used as a positive standard to evaluate anti-MLM antibody activity in the infected sera. This reference standard infected serum was always tested at the same dilutions (from 1/320 to 1/10,240) for its anti-MLM IgM and IgG antibody activity.

Best fitting straight lines were calculated and compared to that obtained with the standard reference serum, which was arbitrarily considered as containing 1000 units of antibody activity per ml (Fig. 1). The dilutions used to calculate the antibody activity units were graphically determined as giving the same ΔOD in the linear and parallel portions of the curves of both the standard and the experimental serum. For serum containing low antibody activity, no linear dose response curve could be observed since, in most cases, a significant binding activity could be obtained only for the lowest dilution tested. For these sera, the dilution chosen for interpolation was the lowest dilution—1/20. When the 1/20 dilution of the infected serum gave a ΔOD less than that obtained with the standard at the highest dilution tested, the antibody activity was considered as null. Thus, for each infected serum the unit of activity was calculated as follows:

$$X \text{ unit} = 1000 \times \frac{\text{reciprocal of dilution of experimental serum}}{\text{reciprocal of dilution of infected serum}}$$

Statistics. Experimental groups of mice

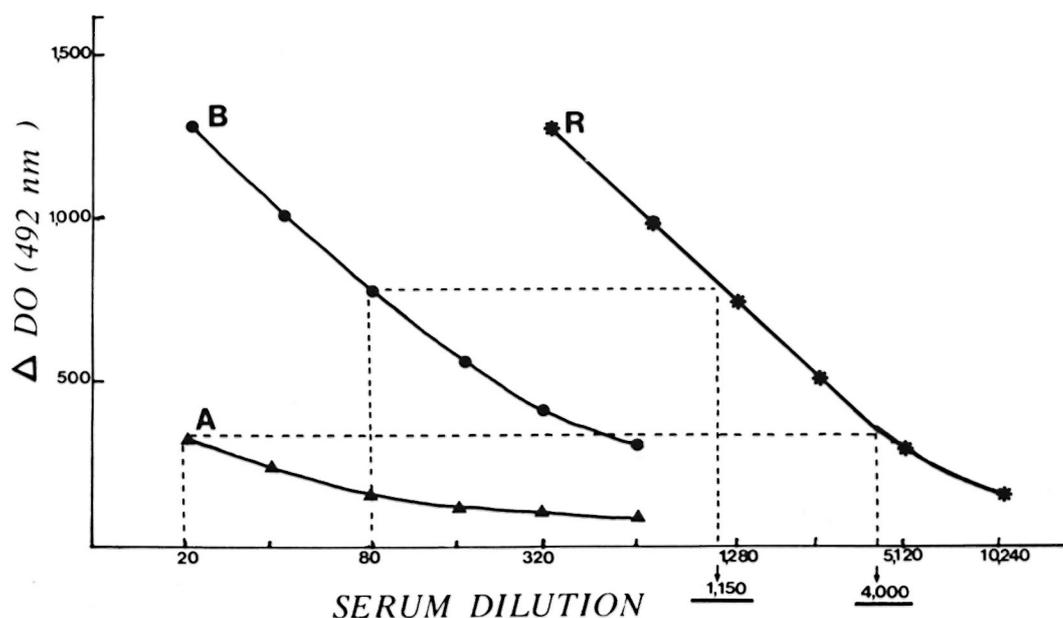


FIG. 1. Mode of calculation of the anti-MLM antibody activity units contained in sera from MLM-infected mice. Standard infected serum R (*) obtained from highly infected mice was arbitrarily defined as containing 1000 antibody activity units per ml. Experimental serum from low-dose-infected mouse A (\blacktriangle) or from high-dose-infected mouse B (\bullet) were analyzed by comparison with the standard infected serum. Anti-MLM antibody activity contained in serum A was calculated as follows: $(20 \times 1000)/4000 = 5$ units per ml; that of serum B: $(80 \times 1000)/1150 = 69.6$ units per ml.

were compared using Student's *t* test or the Mann-Whitney U test. For comparison between IgM and IgG antibody levels, the *t* test for paired data was used. $p < 0.05$ was considered as significant.

RESULTS

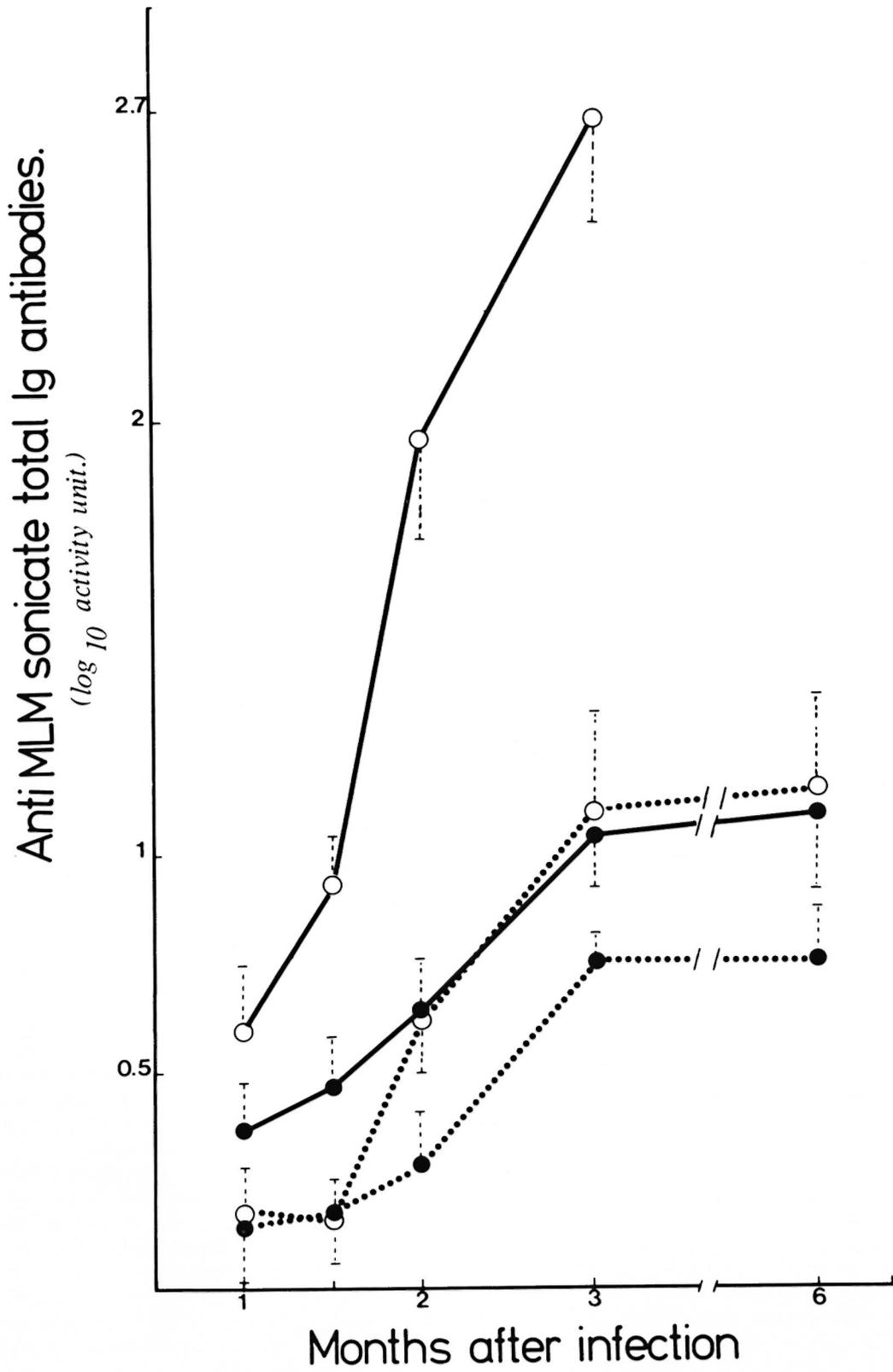
Bacterial load in C57BL/6 mice inoculated i.v. or s.c. with 10^5 or 10^8 MLM. The bacterial load in the spleens of mice infected i.v. and in the foot pads of mice inoculated s.c. was related to the size of the inoculum: the bacillary count was always found two \log_{10} higher in mice inoculated either i.v. or s.c. with 10^8 than with 10^5 MLM (The Table).

In mice infected i.v., the bacterial load in the spleens of those animals inoculated either with 10^8 or with 10^5 MLM showed a sharp and similar increase between 3 and 6 months of infection. Conversely, in mice infected s.c. the bacterial load in the foot pads plateaued during the observation period. No dissemination of bacilli was observed in mice inoculated s.c. regardless of the size of the inoculum, since the bacillary

counts in the spleens did not increase between 3 and 6 months of infection and were similar in mice infected with 10^5 or 10^8 MLM.

Influence of dose and route of inoculation on total anti-mycobacterial antibody formation during course of MLM infection. Antibody production to MLM sonicate was followed during the course of infection of C57BL/6 mice inoculated i.v. or s.c. with two doses of MLM. The time of appearance and the amount of antibodies were dependent on the route and dose of inoculation (Fig. 2). Whatever the route used to inoculate the mice, the anti-mycobacterial antibody responses were stronger and occurred earlier in highly infected mice than in mice infected with a lower dose.

The i.v. route of inoculation induced an earlier and a higher anti-mycobacterial antibody response than did the s.c. route, regardless of the dose used to infect the animals. Thus, when mice were inoculated with 10^8 MLM, a significant antibody response was observed 1 and 2 months after the infection in mice infected i.v. or s.c. ($p < 0.01$,



THE TABLE. Bacterial load in spleens and foot pads of MLM-infected C57BL/6 mice.^a

Bacterial load in	Inoculation		Time of infection	
	Route	Dose	3 mos.	6 mos.
Spleen	i.v.	10 ⁵	5.97 ± 0.48	7.20 ± 0.62
	i.v.	10 ⁸	7.85 ± 0.29	9.16 ± 0.52
Foot pad	s.c.	10 ⁵	6.23 ± 0.34	6.84 ± 0.65
	s.c.	10 ⁸	8.19 ± 0.53	8.59 ± 0.26
Spleen	s.c.	10 ⁵	5.15 ± 0.17	4.98 ± 0.28
	s.c.	10 ⁸	5.30 ± 0.30	5.26 ± 0.38

^a Results are expressed as the geometric mean ± S.D. of log₁₀ of the bacilli count assessed from five individual infected mice per group.

Student's *t* test, as compared to sera from uninfected mice). When mice were inoculated with a lower dose, the appearance of a significant antibody response was delayed in s.c. inoculated mice as compared to mice inoculated i.v. (3 and 2 months, respectively; $p < 0.01$, Student's *t* test, as compared to sera from uninfected mice). At 3 months of infection, antibody levels to MLM sonicate were higher in mice infected i.v. than in mice infected s.c. with the same dose.

Influence of dose and route of inoculation on anti-MLM sonicate IgM and IgG antibody production. The kinetics of appearance and the amount of both IgM and IgG antibodies to mycobacterial antigens were found to depend markedly on the dose used to infect the animals. In high-dose-infected mice, both anti-MLM sonicate IgM and IgG antibody activities were seen earlier and at a stronger level than those observed in low-dose-infected mice.

In mice infected i.v., IgM antibodies to MLM sonicate were produced in higher amounts than IgG antibodies, whatever the dose used to infect the animals (Fig. 3, A & B). A significant difference between the amounts of IgM and IgG antibodies was observed 2 and 3 months after the infection for mice inoculated with 10⁸ or 10⁵ MLM, respectively ($p < 0.05$, *t* test for paired data).

In mice infected s.c., the distribution of the μ and γ isotypes among anti-MLM antibodies was different from that observed in mice infected i.v. Although IgM and IgG antibody levels to mycobacterial antigens showed a similar progression during the period studied, IgG response was always stronger than that of IgM (Fig. 3, C & D). In contrast to mice infected i.v., the influence of the dose of inoculation was only moderate on the amount of IgM antibodies and not significant on IgG antibody production. After a similar inoculum, IgM antibody response was always stronger in mice inoculated i.v. than in mice infected s.c. In contrast, IgG antibody levels were found to be similar, whatever the route of inoculation used to infect the animals.

Correlation of antibody titers with bacillary load. Correlations were calculated between the bacilli numbers enumerated either in the spleens or in the foot pads and the anti-MLM antibody levels, regardless of the time of sacrifice.

In i.v. infected mice, regardless of the dose of inoculation, a similar and significant correlation was found between anti-MLM sonicate antibody levels and the bacillary counts in the spleens of infected mice in all three antibody assays (anti-MLM total Ig: $r = 0.68$, $p < 0.01$; anti-MLM IgM: $r = 0.69$, $p <$

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FIG. 2. Anti-MLM sonicate antibodies in mice infected i.v. and s.c. during the course of MLM infection. C57BL/6 mice were infected i.v. (—) or s.c. (·····) with 10⁵ (●) or 10⁸ (○) MLM. Antibody activity units were calculated as described in Materials and Methods from the total Ig antibody activity contained in the standard reference serum obtained from highly infected mice. The pure standard reference serum was defined arbitrarily as containing 1000 activity units per ml. Results are expressed as geometric mean ± S.E.M. of antibody activity units. The mean ± S.E.M. of background binding antibody activity contained in sera from uninfected mice was equal to 0.14 ± 0.04.

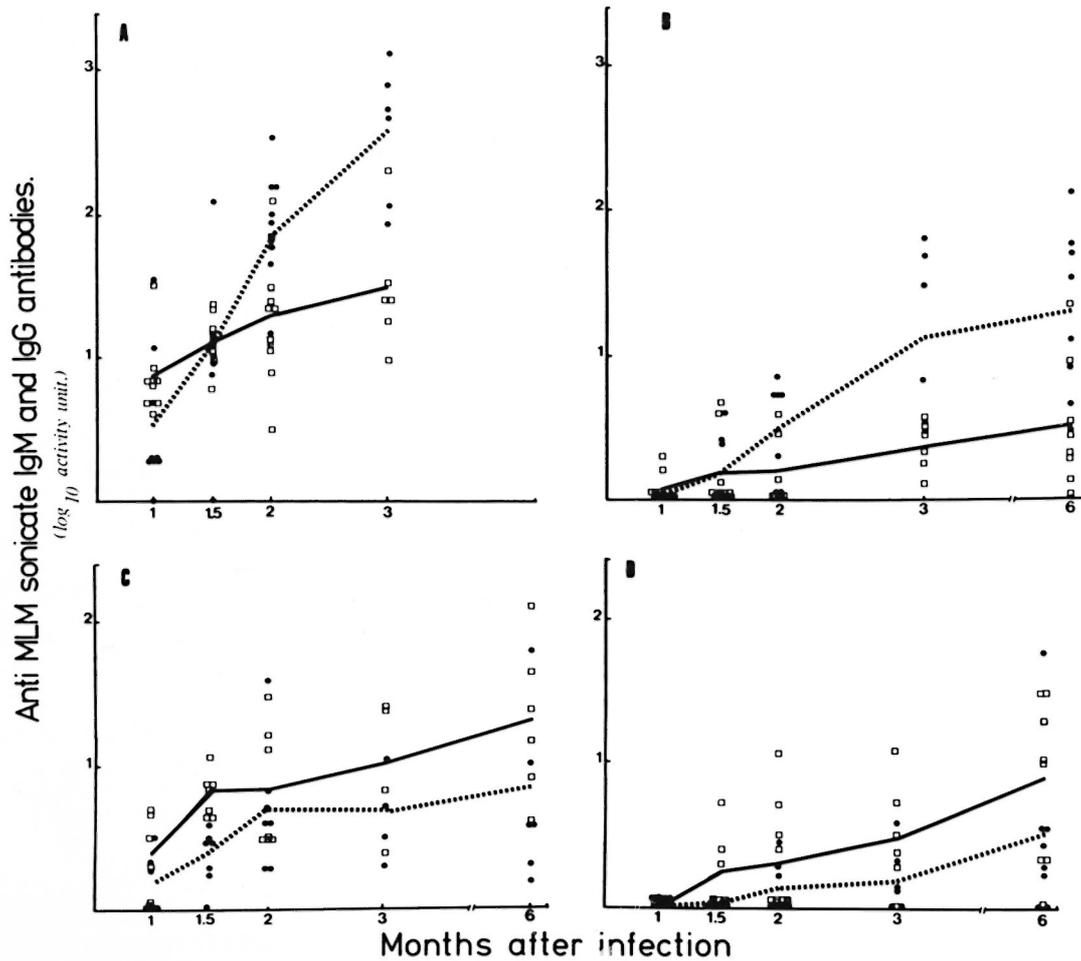


FIG. 3. Anti-MLM sonicate IgM and IgG antibodies according to dose and route of inoculation. A = Mice infected i.v. with 10^8 MLM; B = Mice infected i.v. with 10^5 MLM; C = Mice infected s.c. with 10^8 MLM; D = Mice infected s.c. with 10^5 MLM. Lines represent geometric mean of IgM (●●●) and IgG (—) antibody activity units calculated from the IgM and IgG antibody activity contained in the standard infected serum. Points represent individual serum assessments of IgM (●) or IgG (□) antibodies.

0.01; anti-MLM IgG: $r = 0.68$, $p < 0.01$). (For simplicity, only correlations of bacillary load with both anti-MLM IgM and IgG antibody titers are represented in Fig. 4, A & B.) When the data for the high- and low-dose-infected mice were analyzed separately, a positive correlation was found between IgM antibody levels and the splenic bacterial load ($r = 0.65$, $p < 0.02$; $r = 0.61$, $p < 0.05$ for mice infected with 10^5 and 10^8 MLM, respectively). Conversely, anti-MLM IgG antibody levels did not correlate with the bacterial load. Thus, the apparent correlation of IgG antibody levels to the bacterial load observed when the analysis data

for two different doses were combined might arise from the fact that the IgG antibody level was more related to dose of infection than to number of bacilli per spleen.

Positive correlations were observed among antibody levels measured by the three assays with a stronger correlation between total Ig and IgM antibody titers than between total Ig and IgG ($r = 0.94$, $p < 0.01$ and $r = 0.71$, $p < 0.01$, respectively). IgM and IgG antibody titers also showed a significant correlation ($r = 0.71$, $p < 0.01$).

The increase of anti-MLM antibody titers in relation to the bacterial load was more pronounced for IgM than for IgG antibodies

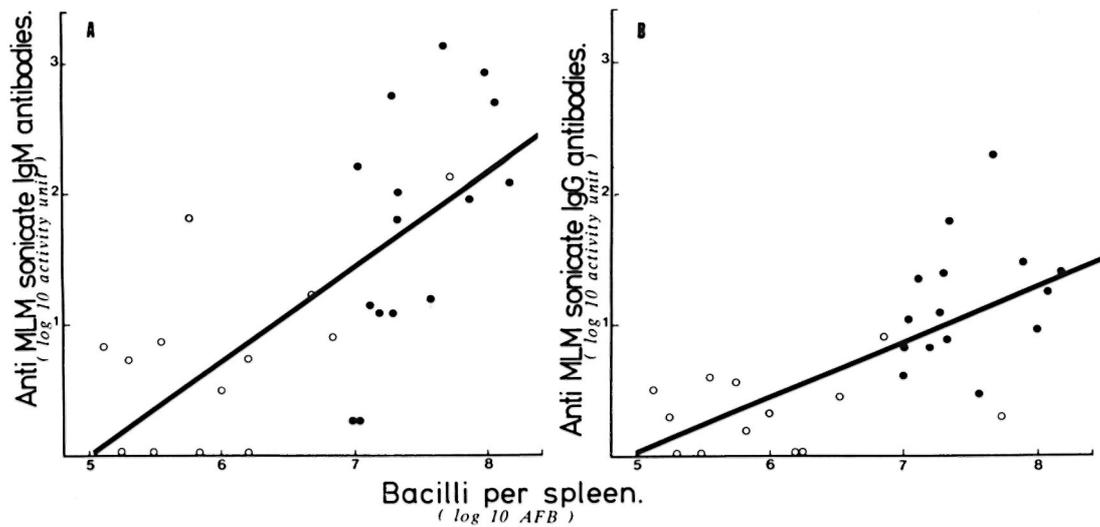


FIG. 4. Correlation of anti-MLM antibody titers to bacterial load in spleens of mice infected i.v. during entire observation period. A = IgM antibodies; B = IgG antibodies. (—) = Best fitting regression lines; O = Mice infected with 10^5 MLM; ● = Mice infected with 10^8 MLM. Correlations between bacilli numbers in the spleens and serum anti-MLM antibody activities were calculated, regardless of the time after infection.

(the slopes of the regression lines were 0.7 and 0.43 for the IgM and IgG responses, respectively).

In s.c. infected mice, significant correlations between antibody levels and bacillary counts in inoculated foot pads were found in the three antibody assays, regardless of the dose of inoculation (anti-MLM total Ig: $r = 0.54$, $p < 0.01$; anti-MLM IgG: $r = 0.57$, $p < 0.01$; anti-MLM IgG: $r = 0.68$, $p < 0.01$). (For simplicity, only correlations of bacterial load with both IgM and IgG antibodies are represented in Fig. 5, A & B.)

Positive correlations were found among antibody levels measured by the three antibody assays: $r = 0.71$, $p < 0.01$ between total Ig and IgM; $r = 0.63$, $p < 0.01$ between total Ig and IgG; and $r = 0.46$, $p < 0.046$ between IgM and IgG. In contrast to i.v. infected mice, the increase of anti-MLM antibody titers in relation to the bacterial loads in the foot pad were found similar for IgM and IgG antibodies (the slopes of the regression lines were 0.23 and 0.33 for the IgM and IgG responses, respectively). It should be noted that the progressive increase in the level of IgM antibodies in relation to the bacterial load was more marked in i.v. than in s.c. infected mice; whereas that of the IgG antibody level remained the same, whatever the route of inoculation.

DISCUSSION

The aim of our present study was to see if the anti-MLM antibody production, and particularly the IgM and IgG antibody levels, could be used as a tool to evaluate the ability to resist MLM infection. Indeed, the C57BL/6 mouse strain was chosen because it has been reported to control the bacillary growth and dissemination when mice were infected s.c.; whereas a widespread dissemination of bacilli occurred when mice were injected i.v. (7, 10, 13, 20). Another point was to determine if the antibody production could serve as an early and valuable indicator of infection. We found that the antibody response appeared earlier and in a greater magnitude in highly infected mice than in mice infected with a lower dose, regardless of the route of inoculation. These results are consistent with those previously reported in mycobacteria-infected mice (6, 16, 21) and in human leprosy where the median antibody titers increased from the tuberculoid to the lepromatous pole of the clinical spectrum (8, 15, 19).

After infection with a similar inoculum, mice inoculated i.v. showed an earlier and a stronger specific antibody response than did mice infected s.c., suggesting the role of either the heavy bacterial load or the bacil-

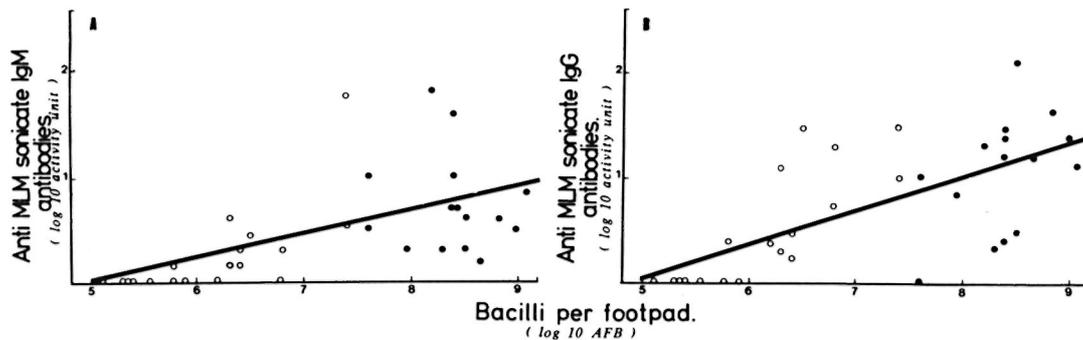


FIG. 5. Correlation of anti-MLM antibody titers to bacterial load in foot pads of mice infected s.c. during entire observation period (see legend for Fig. 4).

lary dissemination on the antibody production. Indeed, the actual roles of either the bacillary dissemination or the bacterial load in antibody production are difficult to elucidate accurately, since i.v. infected C57BL/6 mice showed a bacillary dissemination as well as a heavy bacterial load as the infection progressed. Although the enumeration of bacilli in the spleens of mice infected by the i.v. route only partially reflects the actual bacterial load, it represents an appropriate index of the rate of progression of the whole body bacillary burden⁽¹³⁾. Conversely, C57BL/6 mice infected s.c. controlled the dissemination of bacteria, since they did not exhibit a heavy bacterial burden in their spleens, suggesting that the number of acid-fast bacilli in the foot pads essentially reflects the bacterial load. Therefore, for similar amounts of bacilli evaluated in the spleens and foot pads of mice infected i.v. or s.c., respectively, the actual whole body bacterial load is heavier in i.v. than in s.c. infected mice. This could explain the higher antibody production in mice infected i.v. than that in mice infected s.c.

Anti-MLM IgM and IgG antibody assessments provided further information on the relation between antibody production and the ability to resist MLM infection. Thus, the kinetics of appearance and the amount of anti-MLM IgM and IgG antibodies during the course of infection varied markedly according to the route of inoculation. In i.v. infected mice, IgM antibodies were of a higher magnitude than IgG antibodies. IgM antibody response was found to increase more rapidly with the bacterial load than did the IgG response. In mice

infected i.v., the influence of the size of inoculum and therefore the bacterial load was more pronounced on IgM antibody response than on the IgG response. Moreover, after a similar inoculum mice inoculated i.v. always showed a higher IgM response than did mice infected s.c.; whereas the IgG response was found to be very similar, whatever the route of inoculation used to infect the animals. All of these data strongly suggest that the IgM antibody profile is a better reflection of the dissemination of the bacilli and of a persistent infection than the IgG response.

Similar studies in leprosy patients have also suggested that IgM antibodies to either whole or sonicated *M. leprae* are reliable indicators of persistent infection⁽¹⁹⁾, and are particularly useful markers in the early serodiagnosis of leprosy⁽¹⁴⁾. Species-specific anti-PGL-I antibody assessments emphasize the importance of the IgM response as compared to the IgG response to PGL-I for the early diagnosis of leprosy^(5, 23), the follow up of treatment, and the early detection of relapse⁽³⁾.

Conversely in s.c. infected mice, controlling the bacilli growth and dissemination, IgM and IgG antibodies increased slowly during the first 6 months of infection, with a predominance of IgG antibodies. In these mice, at variance with mice inoculated i.v., the influence of the dose of inoculation was found to be minimal on both IgM and IgG antibody production. However, a stronger correlation of the bacterial load was noted with the IgG response than with the IgM response. Our findings are consistent with those found in tuberculoid patients where

IgG antibodies to whole *M. leprae* could be a sign of activity of the disease (¹⁹). Thus, the ability to control mycobacterial infection in both mice and leprosy patients might be associated with a stronger level of IgG than IgM antibodies (^{3, 14, 19}). High production of IgG antibodies might reflect the activated state of helper-T lymphocytes in tuberculoid patients and in mice controlling MLM infection.

Our present findings were observed in the C57BL/6 mouse strain and should not be extrapolated to other mouse strains. In fact, we have previously reported a striking strain variation in anti-MLM antibody production in spite of a similar bacterial load (¹¹). In human leprosy, wide variations in antibody content in individual sera from patients with a similar clinical classification have been extensively reported (^{3, 8, 14, 23}). Moreover, in both a few untreated lepromatous patients (⁴) and in certain heavily infected mouse strains (^{2, 9}), poor production of anti-mycobacterial antibodies has been described, suggesting a genetic immunological unresponsiveness. All of these data strongly suggest, both in leprosy patients and in MLM-infected mice, a genetic control of anti-mycobacterial antibody production that has recently been demonstrated on the anti-PGL-I antibody response in various mouse strains immunized with *M. leprae* (¹⁸).

The type of antibody assays, and particularly the type of antigen, used in leprosy studies favors the showing up of either the IgM or the IgG response. In our study we analyzed the anti-MLM antibody response with sonicated MLM as antigen, which represents a complex mixture of lipids, polysaccharides, and proteins. The response elicited varies with the antigens used and may be different with a more defined antigen. Thus, the *M. leprae*-specific antigen PGL-I was demonstrated to induce preferentially IgM antibody response (²³). Therefore, since different results can be obtained depending on the type of antibody assay used, antibody measurements have to be considered carefully in relation to the purpose of antibody studies in leprosy.

Our findings suggest that a dominant IgM response to mycobacterial antigens might be a valuable indicator of persistent infec-

tion, especially in disseminated infection, and that a low but predominant IgG response would represent a sign of controlled infection. However, noticeable individual variations in antibody production strengthen the importance of simultaneous assays for IgM and IgG antibodies to mycobacterial antigens in serial samples to identify single individuals at an early stage of infection and to assess the level of infection.

SUMMARY

Mice of the C57BL/6 strain were injected either subcutaneously (s.c.) in the foot pad or intravenously (i.v.) with 10^5 or 10^8 *Mycobacterium lepraemurium* (MLM). Anti-MLM sonicate total immunoglobulin (Ig), IgM, and IgG antibody production was followed during the course of the infection. The kinetics of appearance and the magnitude of anti-MLM antibodies were found to be related to the size and route of inoculation. The i.v. route induced earlier and higher amounts of anti-MLM antibodies than did the s.c. route. In i.v.-infected mice, a relatively predominant IgM response to MLM was observed, while a relatively higher IgG response was seen in s.c.-infected mice. IgM antibody level was found to increase sharply with the bacterial load as assessed in the spleens of mice with i.v.-disseminated infection; whereas a slow progression of both IgM and IgG levels was noted with time in s.c.-infected mice.

RESUMEN

Se inyectaron ratones C57BL/6 subcutáneamente (s.c.) en la almohadilla plantar o intravenosamente (i.v.) con 10^5 ó 10^8 *Mycobacterium lepraemurium* (MLM). Durante el curso de la infección se midió la producción de anticuerpos (Ig total, IgM e IgG) contra un sonicado de MLM. La cinética de aparición y la magnitud de la respuesta en anticuerpos anti-MLM estuvieron relacionadas con el tamaño de la dosis y la ruta de inoculación. La ruta i.v. indujo una respuesta anti-MLM más temprana y más elevada que la inoculación s.c. En los ratones infectados i.v. se observó una respuesta predominantemente del tipo IgM, mientras que en los animales inoculados s.c. se observó una respuesta tipo IgG relativamente más elevada. Se encontró que el nivel de anticuerpos IgM aumentó marcadamente y en forma proporcional a la carga bacteriana medida en el bazo de los animales inoculados i.v. con infección disseminada; en los animales infectados s.c. se observó

una lenta progresión en los niveles tanto de IgM como de IgG.

RÉSUMÉ

On a inoculé des souris de la souche C57BL/6, soit par voie sous-cutanée dans le coussinet plantaire, soit par voie intraveineuse, avec 10^5 ou 10^8 de *Mycobacterium lepraemurium* (MLM). La production des anticorps, immunoglobulines totales (Ig), IgM, et IgG, ont été suivies dans un sonicat anti-MLM au cours de l'infection. On a observé que la cinétique du moment de l'apparition, et la quantité d'anticorps anti-MLM, étaient en relation avec la dose d'inoculation, ainsi qu'avec la voie utilisée. La voie intraveineuse a entraîné la production plus précoce de quantités plus élevées d'anticorps anti-MLM, que ne l'a fait la voie sous-cutanée. Chez les souris infectées par voie intraveineuse, on a relevé une prédominance relative de la réponse IgM au MLM, alors qu'une réponse relativement plus forte des IgG était constatée chez les souris infectées par la voie sous-cutanée. Les taux d'anticorps IgM augmentaient rapidement avec la charge bactérienne, ainsi qu'on a pu le mettre en évidence dans les rates de souris présentant une infection disséminée à la suite d'une dissémination intraveineuse. Par contre, la progression des taux d'IgM et d'IgG était lente chez les souris infectées par voie sous-cutanée.

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