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Experimental leprosy in monkeys. II. Longitudinal serological observations in sooty mangabey monkeys

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Summary In this study, 11 SMM were grouped and inoculated with differing doses of SMM-origin *Mycobacterium leprae* (ML) between $4{\cdot}5 \times 10^8$ and 1×10^9 by either combined IV/IC routes or by IV or IC route alone. The combined route was the most effective in eliciting progressive, disseminated LL leprosy. In all, 6 of 7 SMM inoculated by the combined routes developed leprosy requiring treatment at some point. Only 1 of 4 inoculated by a single route developed persisting leprosy requiring chemotherapy. Either no disease or spontaneous regression of initial disease occurred in the other 3 animals inoculated by a single route. Doses in excess of 1×10^9 ML were more effective than lesser doses.

An association was observed between the development of IgG anti-PGL-I ELISA OD values and resistance to leprosy and between IgM anti-PGL-I and leprosy progression or susceptibility. Serum PGL-I antigen levels, determined by dot ELISA, paralleled disease severity longitudinally. High positive OD values of anti-LAM IgG prior to ML inoculation were observed in the majority of leprosy-susceptible SMM in contrast to negative levels in more resistant animals. Anti-LAM IgG OD values exceeded the positive cut-off point after inoculation in 5 of 11 SMM; 3 of these 5 had concurrent detectable serum levels of PGL-I antigen.

Introduction

We previously observed that longitudinal serologic data from sooty mangabey monkeys (SMM) (*Cercocebus torquatus atys*) experimentally inoculated with *Mycobacterium leprae* (ML) may provide indicators for susceptibility to leprosy.[1,2] In that study,

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Table 1 Inoculations and results

M. leprae inoculated ($\times 10^{-8}$) Animal	IV	IC	total	Disease description and characteristics	Degree of susceptibility
E045	4·5	6.2	10·7	LL, disseminated	susceptible
E042	4·5	5·9	10·4	no disease	resistant
E043	4·5	6·2	10·5	LL, disseminated	susceptible
E044	4·5	6·0	10·5	LL, disseminated	susceptible
E038	0·04	6·0	6·04	BB-BL, progressive	susceptible*
E039	0	6·0	6·0	LL, self-healed	resistant
D215	0	6·0	6·0	no disease	resistant
E040	4·5	0·06	4·56	LL_s, neural	susceptible*
E047	4·5	0·06	4·56	LL, disseminated	susceptible
E041	4·5	0	4·5	no disease	resistant
E046	4·5	0	4·5	BL-LL, progressive	susceptible

* SMM E038 and E040 each showed some degree of resistance, including repeated episodes of spontaneous regression, followed by later progression. In E038, leprosy in the BB-BL or BB region was observed; E040 initially developed BL-LL, but eventually developed LL_s, primarily involving the nerves, but at some few sites there were also indications of dermal disease. These 2 SMM were, therefore, designated as 'susceptible with resistance'.

SMM were inoculated by combined intravenous (IV) and intracutaneous (IC) routes with titrated doses of SMM-origin ML and studied longitudinally by ELISA for IgG and IgM antibody (Ab) levels to phenolic glycolipid-I (PGL-I) antigen (Ag) and lipoarabinomannan (LAM) Ag. The prior data suggested that the ratio of the levels of IgM : IgG anti-PGL-I determined by ELISA were elevated in animals that were susceptible to lepromatous (LL) leprosy. By contrast, the IgM : IgG anti-PGL-I ELISA ratios were low in SMM that were resistant to LL leprosy. A longitudinal shift from low to high ratios of IgM : IgG anti-PGL-I OD values predicted and/or coincided with advancement of disease to a LL form.[1] Similar relationships were subsequently observed in 2 chimpanzees with naturally-acquired BL–LL leprosy studied longitudinally.[3]

The prior data also suggested that high preinoculation ELISA levels of anti-LAM IgG correlated positively with susceptibility to LL leprosy when compared to SMM with low or negative anti-LAM IgG levels.[2] Positive anti-LAM IgM levels were observed only in SMM with advanced, disseminated LL leprosy.[2]

In the present study we inoculated additional SMM with differing doses of SMM-origin ML using combined IV/IC routes. In addition, some SMM were inoculated with similar doses of ML by IV or IC routes alone. Longitudinal sera were assayed by ELISA for anti-PGL-I and anti-LAM IgG and IgM levels and correlated with clinical leprosy findings. Also, PGL-I Ag levels in sera were determined in parallel longitudinally by dot-ELISA.[4,5] Relationships between ELISA-determined IgM and IgG Ab proportions, PGL-I Ag levels and disease progression were determined.

Materials and methods

Inoculations: Preparation of SMM-origin ML and the method of combined IV/IC inoculations are described in Part I of this study.[9] Briefly, IV inoculation was via the

saphenous vein and IC sites were: 2 sites on each ear margin, the tip of the nose, an outer forearm, an outer calf and sometimes the periorbital regions.

Animals were inoculated and clinical characteristics noted (Table 1). Based on the clinical observations, the animals were subdivided into 3 groupings describing their apparent degrees of susceptibility to leprosy (Table 1).

Animals were examined 3–4 times per year with some exceptions, as noted in the text, and the degree of advancement over time was staged as previously reported.[1] Briefly, the staging system is: −, no disease; ±, nonulcerated areas (< 2 mm) of abnormal pigmentation or/and infiltration at dermal inoculation sites; 1+, well stained AFB in nasal secretions or in a biopsy specimen or >2 mm lesions at multiple inoculation sites; 2+, AFB in nasal secretions and in lesions at inoculation sites; 3+, lesions at uninoculated sites; and 4+, ulceration or enlargement (>1 cm) of or increases in numbers or disseminated dermal leproma.[1] Regressive episodes are defined as periods during which the staging criteria diminished in severity over time. Histopathologic classifications, lepromatous (LL), borderline lepromatous (BL), borderline (BB), indeterminate (Ind) or neuritic, were according to the Ridley–Jopling system based on evaluations of H & E and Fite–Faraco-stained biopsy specimens.[6–8] Details of clinical outcome of leprosy are described in Part I of this study.[9]

Baseline sera were obtained prior to ML inoculations and at intervals after inoculation and were stored frozen for later ELISA evaluations of anti-PGL-I or anti-LAM Ab and for determination of PGL-I Ag levels by a dot-ELISA method.[4,5]

ELISA: The assays were performed as previously reported.[1–3] Natural ML PGL-I and *M. tuberculosis* LAM were used as Ags (specificity for A-PGL-I was verified using the synthetic glycoconjugate, bovine serum albumin-O-(3,6-di-O-methyl–beta-D-gluco-pyranosyl)-(1–4)-(1-deoxy-2,3-di-O-methyl-L-rhamnose) (NDO-BSA), instead of natural PGL-I in all animals; only the natural PGL-I data are reported). PGL-I, LAM and NDO-BSA were provided by Dr Patrick J. Brennan, Colorado State University School of Veterinary Medicine, Fort Colline, CO under NIH contract No 1-AI-52582.

Briefly, 96-well plates were coated with Ag, washed, blocked with BSA, washed again and reacted with a previously determined optimal dilution of monkey serum. After incubation and washing, the plates were coated with peroxidase-labeled anti-human IgG or IgM Fc fragment γ- or μ-chain-specific Ab diluted according to prior titrations, incubated, washed, reacted with o-phenylenediamine + H_2O_2, acidified and ODs determined at 490 nm on an ELISA reader.[1,2] Final ODs represent the difference in absorbance between wells containing Ag minus wells lacking Ag but containing all other components. Each reagent in the ELISA was carefully titrated in a checkerboard manner to determine dilutions that would give final OD values between 0·1 and 0·5 OD whenever possible to utilize the OD range most sensitive to small changes in OD so that small changes from sample to sample would have maximal meaning and would accurately reflect longitudinal changes. All sera were assayed together once in given experiments to permit accurate relative comparisons. The same batch of peroxidase antibody was used throughout. All experiments were repeated on at least 2 separate occasions examining all sera together in each assay. OD values obtained with these precautions were reproducible in a given sample from 1 assay to another to within ±0·05. The data presented represent results from a single occasion. The following values were determined for normal SMM (mean ± 1 SD, $n = 101$): anti-PGL-I IgG, 0·011 ± 0·016; anti-PGL-I IgM, 0·019 ± 0·027; anti-LAM IgG, 0·118 ± 0·103; and anti-LAM IgM, 0·026 ± 0·049. The

mean + 2 SD, taken as the cut-off points, were for IgG and IgM, respectively, 0·043 and 0·073 (anti-PGL-I) and 0·324 and 0·124 (anti-LAM).

Detection of PGL-I antigen in sera: PGL-I antigen detection procedures have been described in detail previously.[4,5] Briefly, for serum lipid extraction, 100 μl of serum was added to filter paper discs ($\frac{1}{2}''$ in diameter) and dried completely. Lipids were then extracted using 2–3 ml of $CHCl_3 : CH_3OH$ (2 : 1) solution and dried under N_2. Serum lipids were dissolved in $CHCl_3$ and applied to fluorosil packed in a pasteur pipette, 60–100 mesh (Sigma Chemical Co., St Louis, MO, USA) and eluted with $CHCl_3$, followed by 5% CH_3OH in $CHCl_3$. The lipid fraction eluted with 5% CH_3OH was saved and dried under N_2 and examined for the presence of PGL-I by a dot-ELISA method as previously reported.[4,5] The lipid fraction was dissolved in 100 μl of hexane and a 5 μl portion was applied to a tuffryn (polysulphone) membrane (HT-200) (Gelman Sciences Inc., Ann Arbor, MI, USA), followed by anti-PGL-I antibody. A high titre of rabbit anti-PGL-I antibody (a gift from Dr P. J. Brennan) was used for the primary antibody and peroxidase-conjugated goat anti-rabbit IgG (Cooper Biomedical Inc., Malvern, PA, USA) was used as the secondary antibody. For colour development, 4-chloro-1-naphthol (Biorad Laboratories, Richmond, CA, USA) was used and the results were read visually.

Results

The longitudinal ELISA anti-PGL-I and anti-LAM IgG and IgM data were plotted in parallel with the longitudinal PGL-I serum Ag level and the degree of clinical disease (Figures 1–11). The ± stage of disease is not indicated on the graphs, i.e. it shows as zero. The clinical progress of leprosy in these 11 SMM is described in detail in Part I of the Study.[9] In all, 3 of 4 SMM given approximately $10{\cdot}5 \times 10^8$ ML ('high' dose) by combined IV/IC routes (E045, E043 and E044—Figures 1, 3 and 4) developed progressive (i.e. advancing visible clinical symptoms), disseminating (i.e. lesions appearing at uninoculated sites) LL leprosy concurrently with elevated levels of serum PGL-I Ag and were designated as leprosy susceptible. The remaining SMM in this group, E042, developed no disease, no serum PGL-I Ag was detectable and was designated resistant (Figure 2). Ratios of anti-PGL-I IgM : IgG ELISA readings in E045, E043 and E044 were >1 during the period of advancing disease and high PGL-I Ag loads (Figures 1, 3–4).

The PGL-I IgM : IgG ratio became elevated in excess of 1 in all 11 SMM immediately PI and remained >1 for up to 5 months PI in some cases. As a result, the IgM : IgG ratio data during the first 5 months PI is not a reliable indicator of the future course of the disease. Thus, the IgM : IgG ratios over the first 5 months PI will not be considered.

Ratios of anti-PGL-I IgM : IgG were <1 and no detectable PGL-I Ag was present throughout the period of study in E042, a leprosy-resistant SMM that received a high ML dose by combined IV/IC routes (Figure 2). A similar pattern of the ratio of IgM : IgG A-PGL-I being <1 with no detectable PGL-I serum Ag was also observed in other leprosy-resistant SMMs that received lower doses of ML by a single route (E039 and D215, 6×10^8 ML by IC route only, Figures 6 and 7). Another leprosy-resistant SMM, E041 (Figure 10), had relatively low and variable levels of IgG- and IgM-A-PGL-1 Ab which sometimes exceeded the cut-off points. This low and fluctuating Ab level

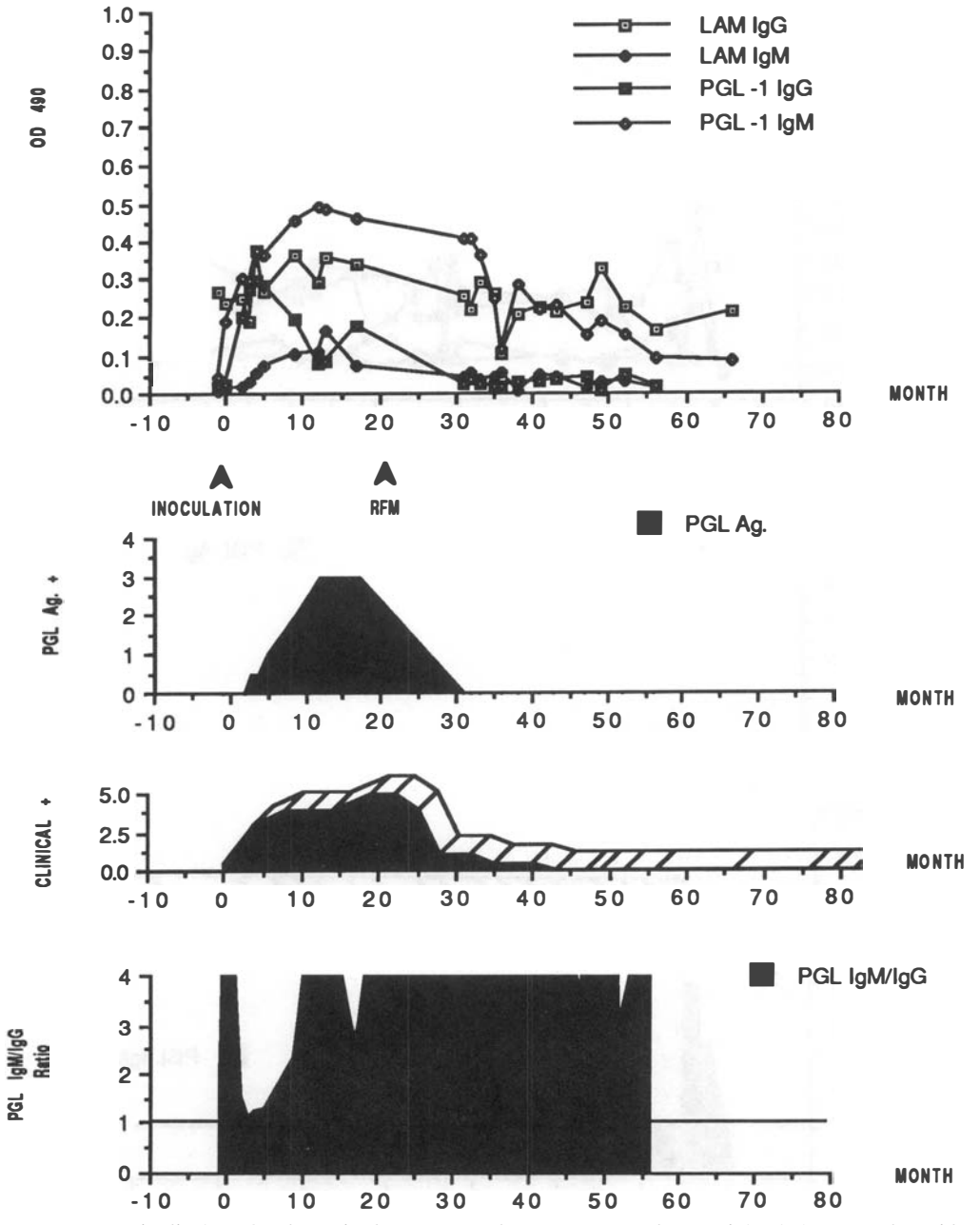

Figure 1. Longitudinal ELISA-determined A-PGL-I and A-LAM IgG and IgM Ab levels (top) together with serum PGL-I Ag levels (top middle), clinical staging of leprosy (bottom middle) and the ratio of ELISA-determined A-PGL-I IgM : IgG (bottom), leprosy-susceptible SMM E045, experimentally inoculated IV with $4{\cdot}5 \times 10^8$ and IC with approximately 6×10^8 ML.

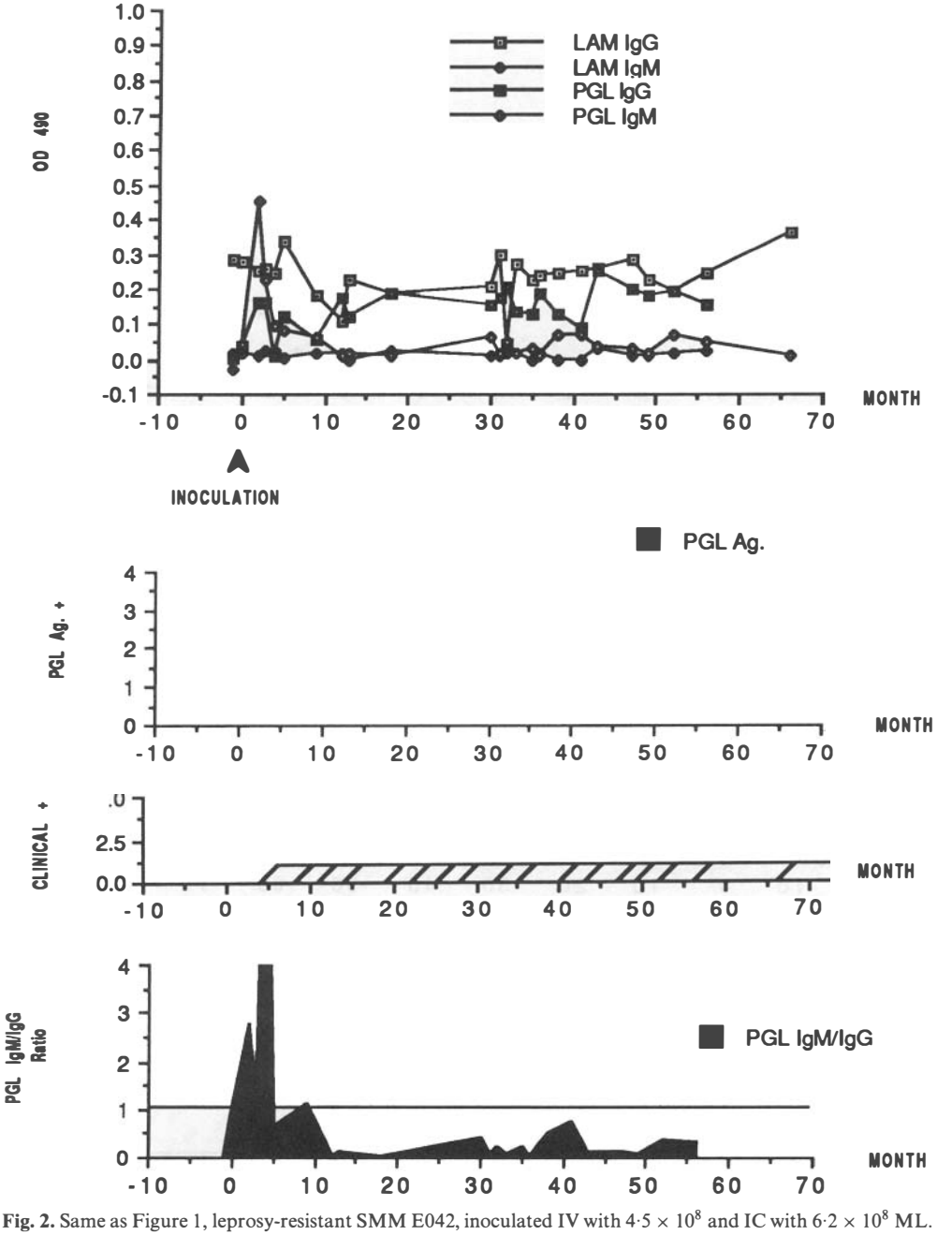

Fig. 2. Same as Figure 1, leprosy-resistant SMM E042, inoculated IV with $4{\cdot}5 \times 10^8$ and IC with $6{\cdot}2 \times 10^8$ ML.

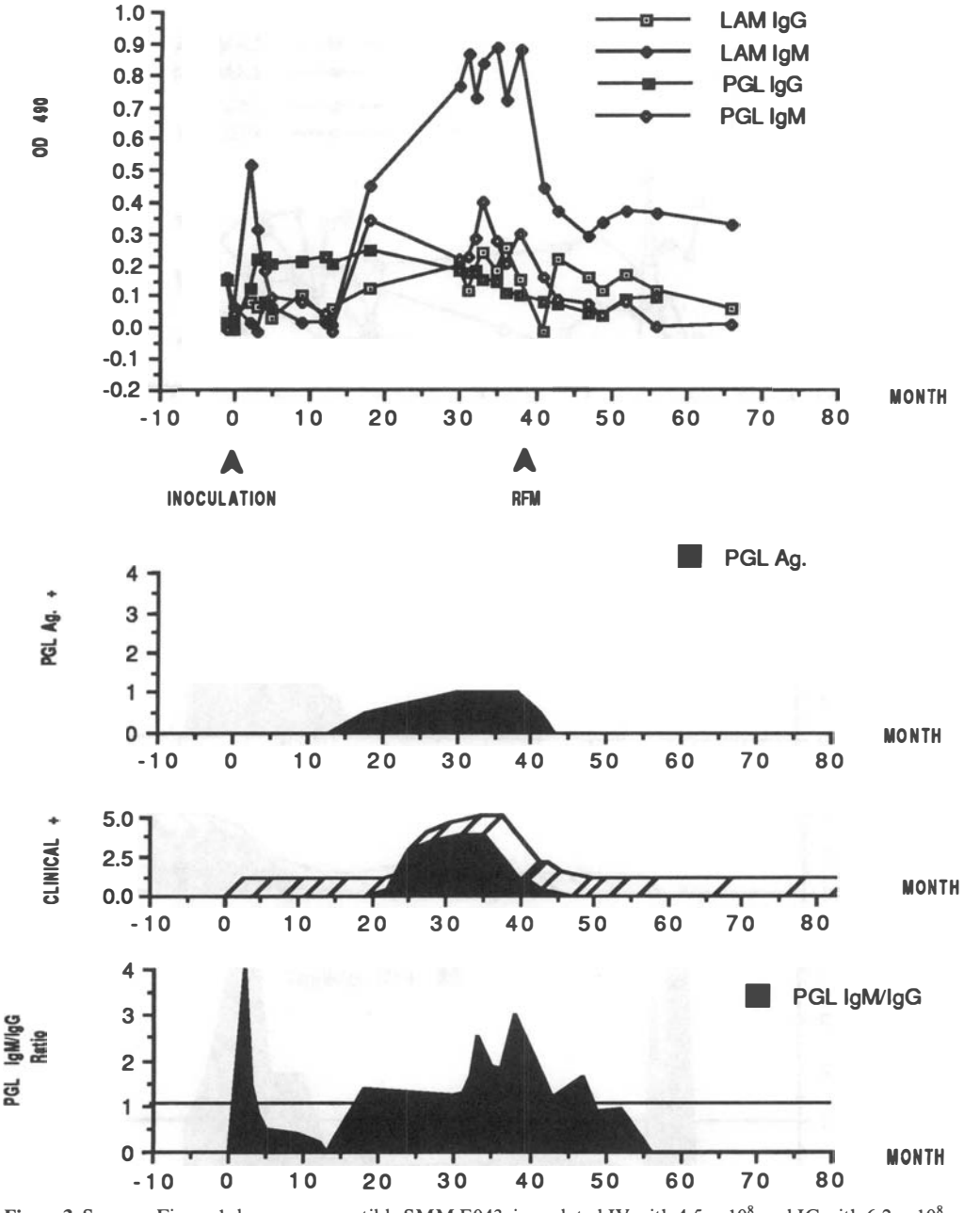

Figure 3. Same as Figure 1, leprosy-susceptible SMM E043, inoculated IV with $4{\cdot}5 \times 10^8$ and IC with $6{\cdot}2 \times 10^8$ ML.

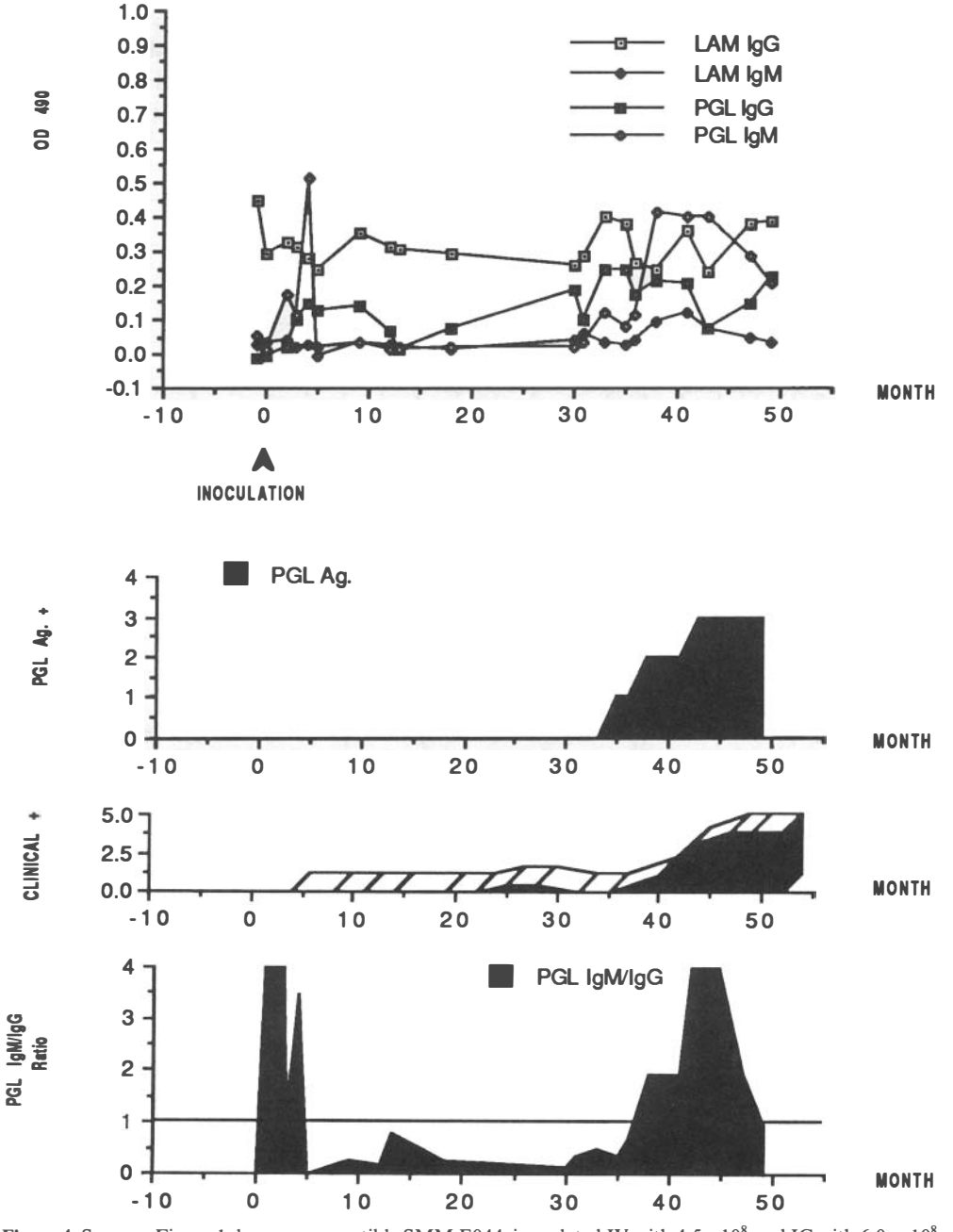

Figure 4. Same as Figure 1, leprosy-susceptible SMM E044, inoculated IV with $4{\cdot}5 \times 10^8$ and IC with $6{\cdot}0 \times 10^8$ ML.

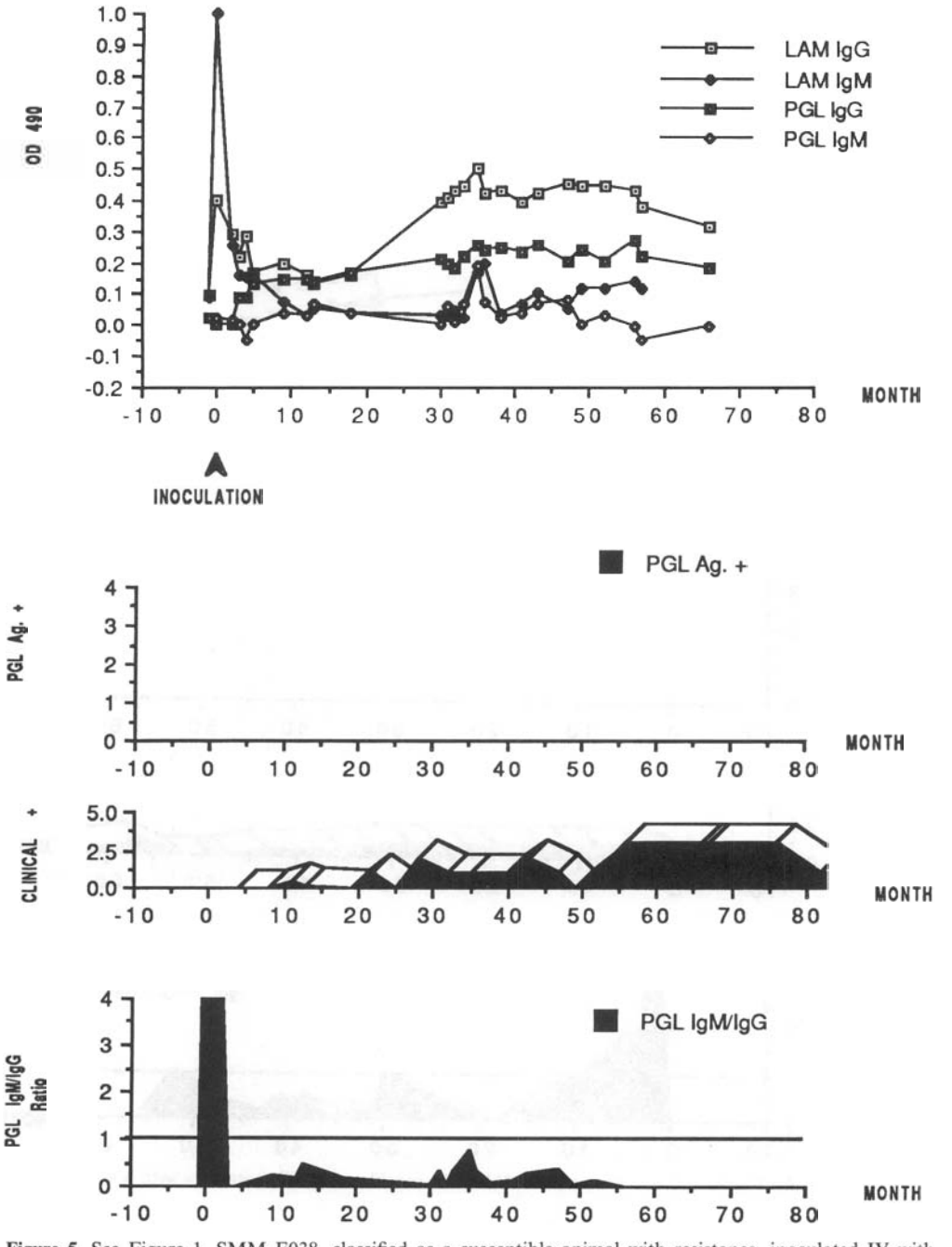

Figure 5. See Figure 1, SMM E038, classified as a susceptible animal with resistance, inoculated IV with $4{\cdot}0 \times 10^6$ and IC with 6×10^8 ML.

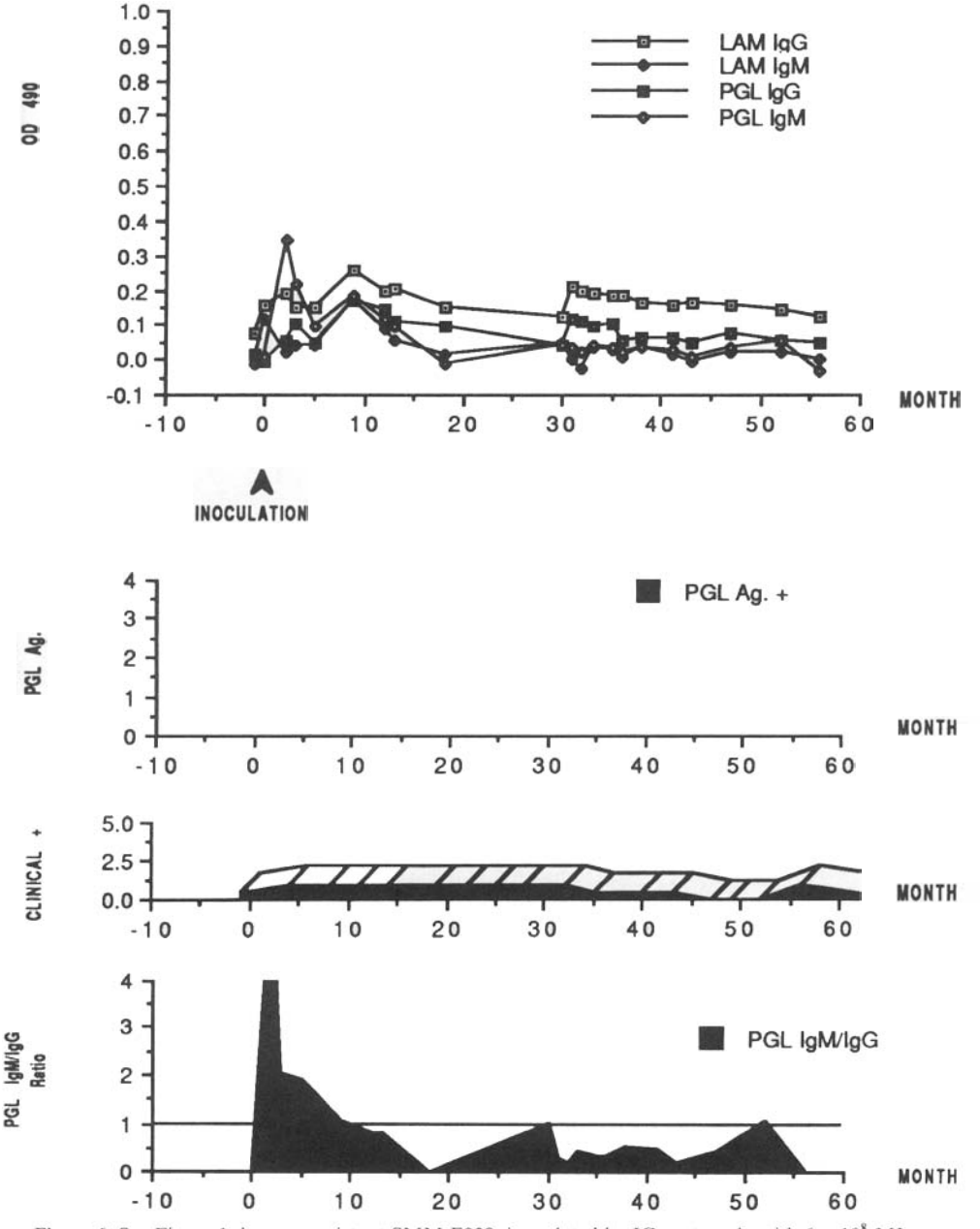

Figure 6. See Figure 1, leprosy-resistant SMM E039, inoculated by IC route only with 6×10^8 ML.

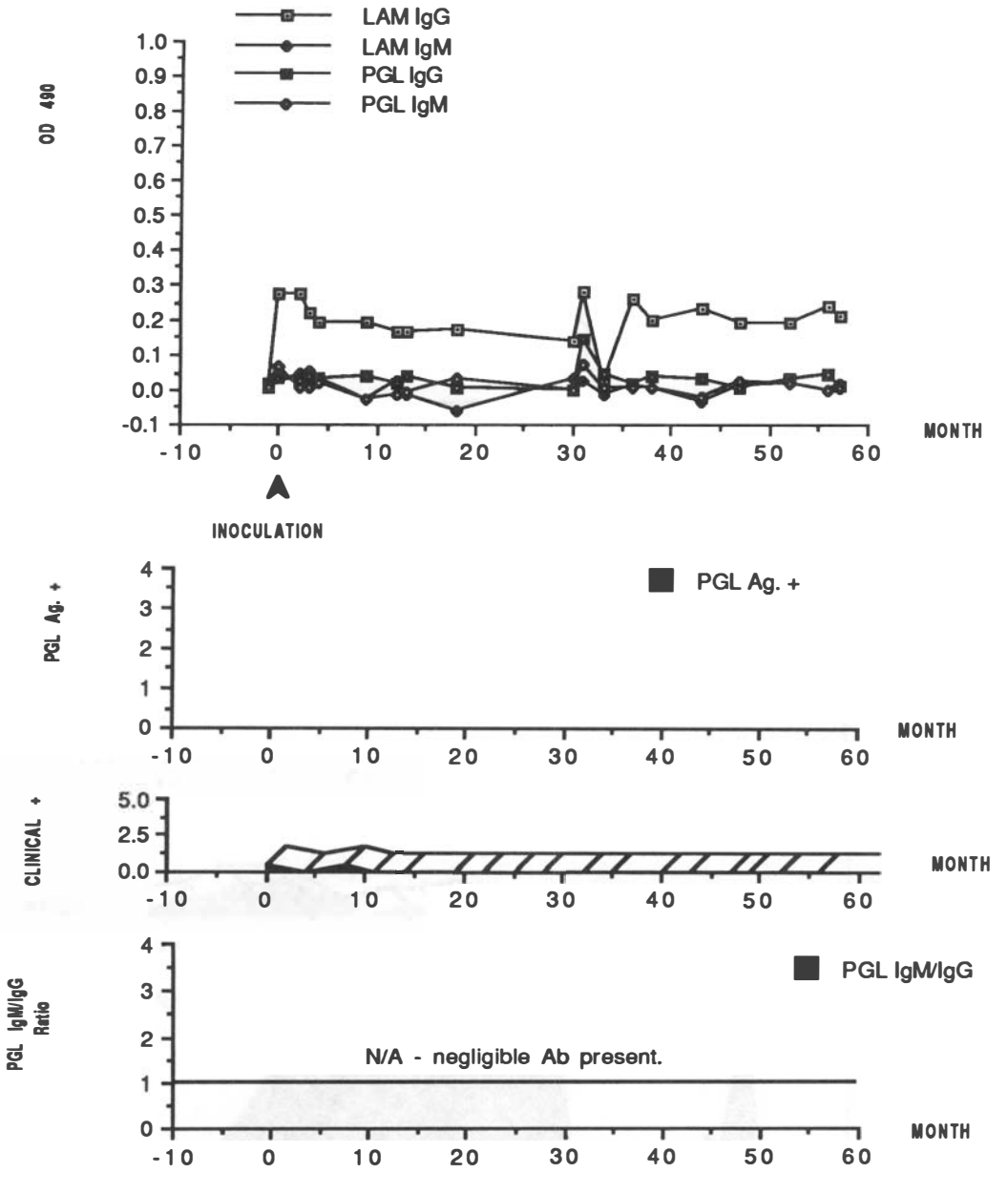

Figure 7. Same as Figure 6, leprosy-resistant SMM D215, inoculated by IC route only with $6{\cdot}0 \times 10^8$ ML.

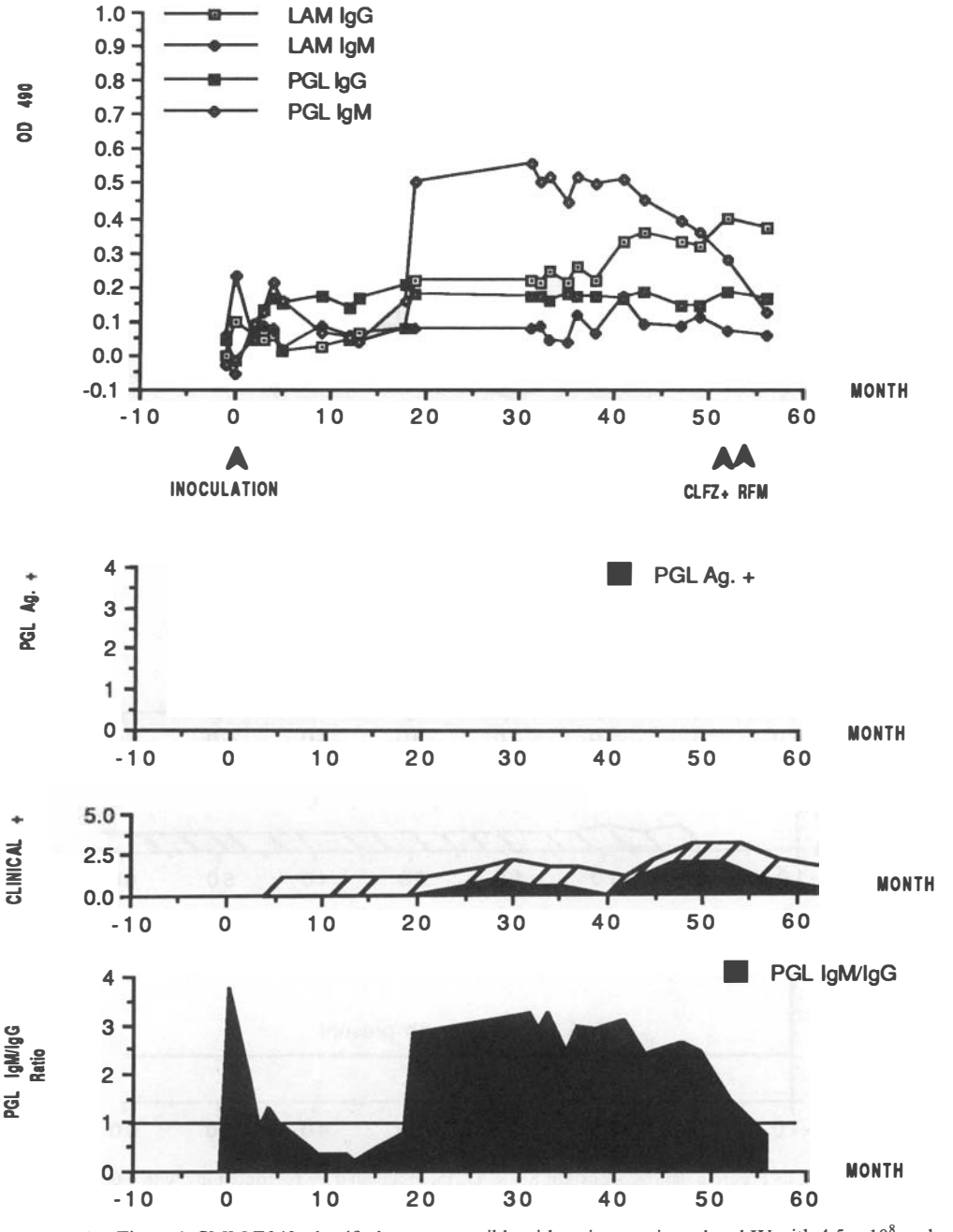

Figure 8. See Figure 1, SMM E040, classified as a susceptible with resistance, inoculated IV with $4{\cdot}5 \times 10^8$ and IC with 6×10^8 ML.

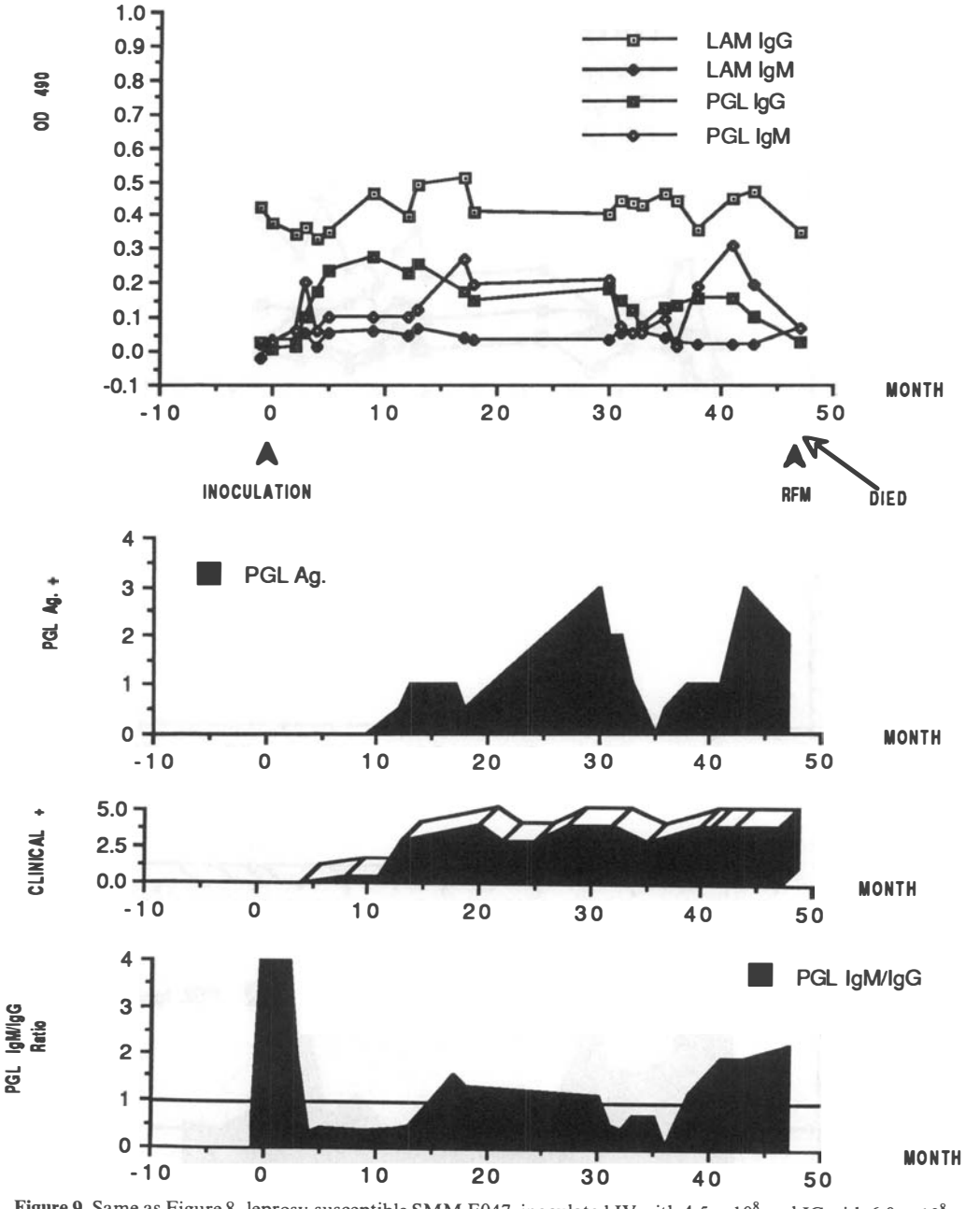

Figure 9. Same as Figure 8, leprosy-susceptible SMM E047, inoculated IV with $4{\cdot}5 \times 10^8$ and IC with $6{\cdot}0 \times 10^8$ ML.

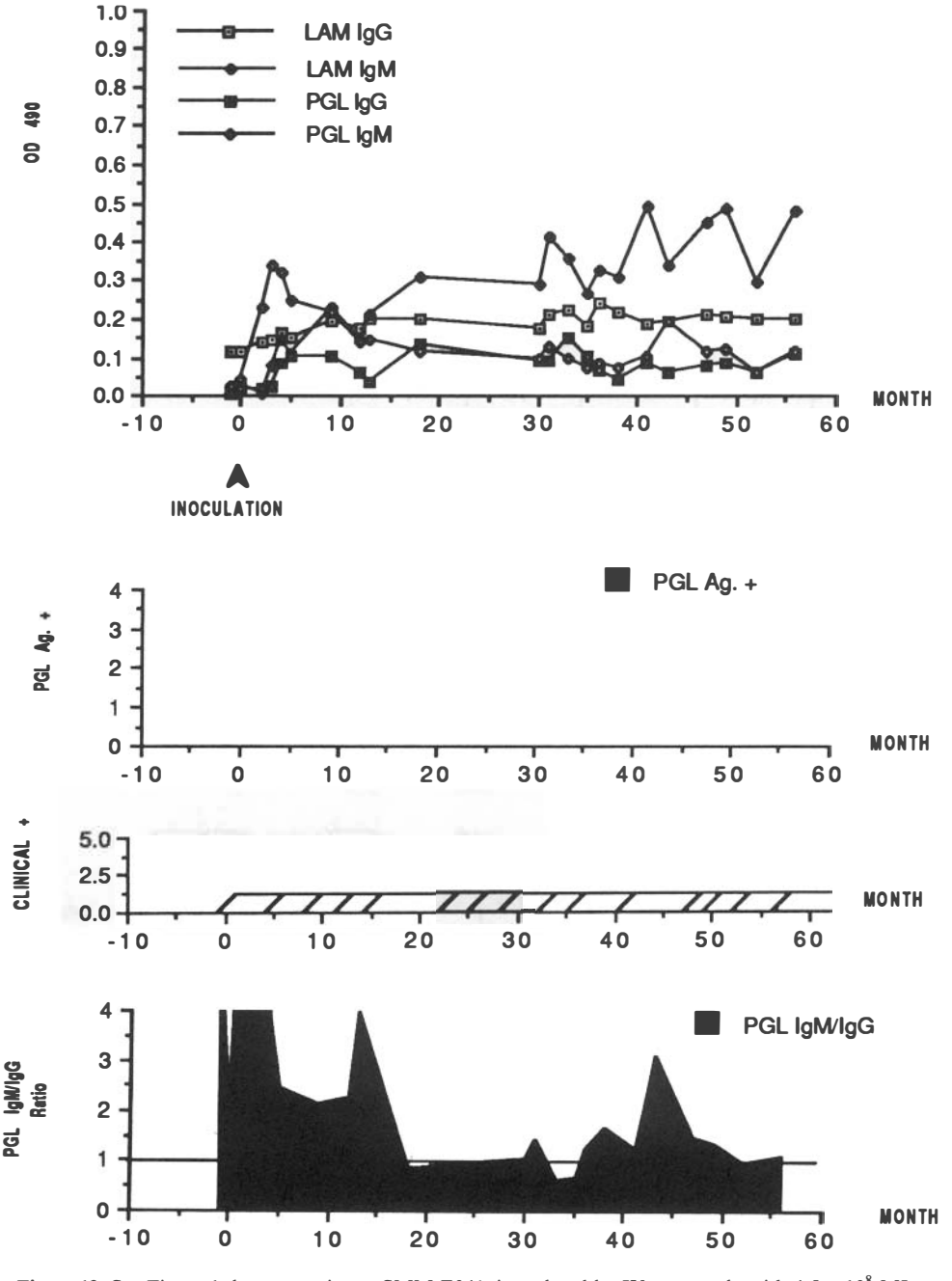

Figure 10. See Figure 1, leprosy resistant SMM E041, inoculated by IV route only with $4{\cdot}5 \times 10^8$ ML.

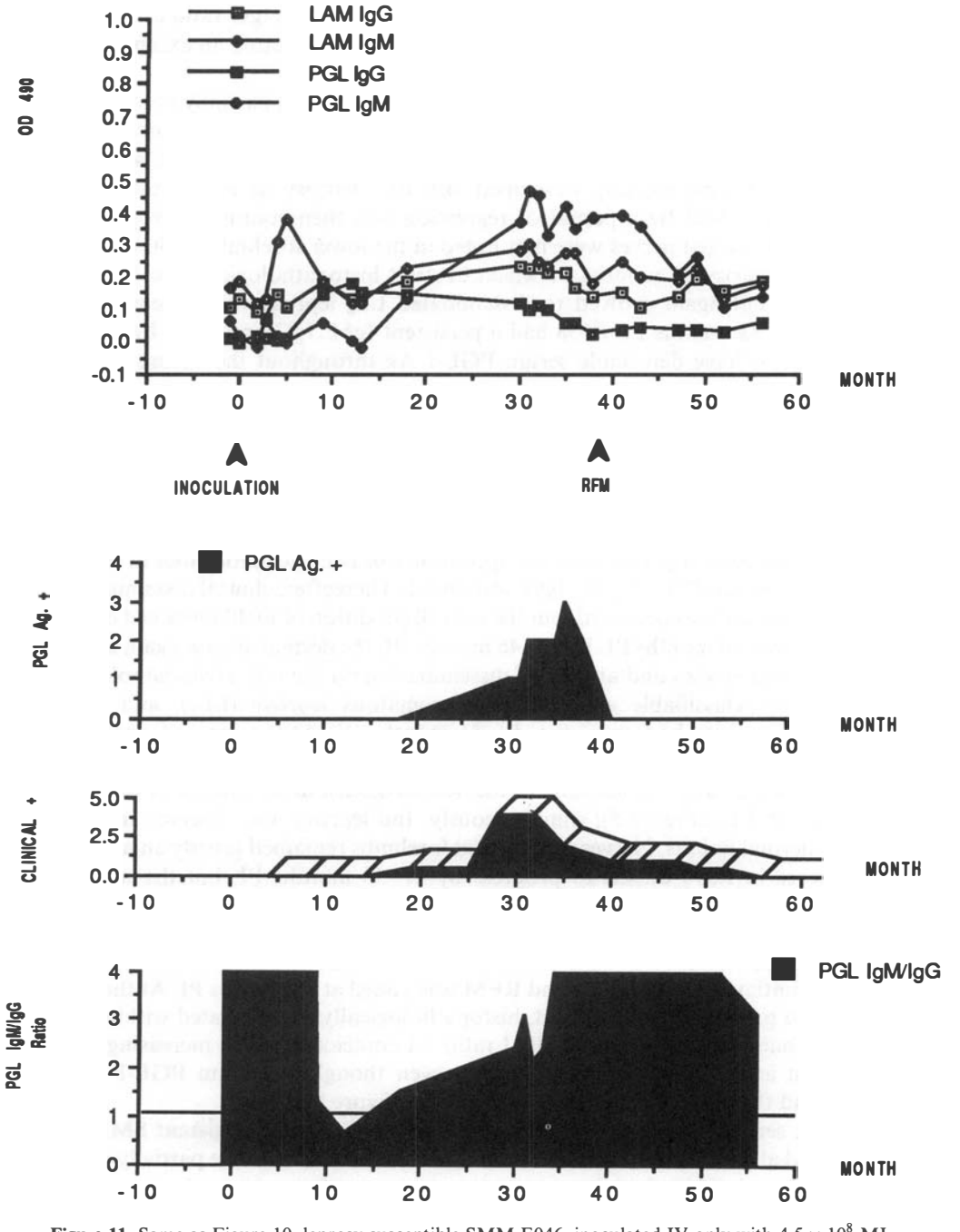

Figure 11. Same as Figure 10, leprosy-susceptible SMM E046, inoculated IV only with $4{\cdot}5 \times 10^8$ ML.

made the ratio calculation appear misleading in that the IgM : IgG ratio exceeded 1 at least once in E041 even though no disease was visible, representing an exception to the observations.

SMM E038 (Figure 5, Table 1) was a progressive/regressive, partially resistant SMM designated susceptible with resistance (it had multiple episodes of spontaneous regression followed by further progression). E038 was inoculated with 6×10^8 IC and 4×10^6 IV ML (Table 1) and initially developed BB–BL[9] leprosy at inoculation sites by 22 months PI followed by a period of regression and then continued progression at 26 months PI. Enlarged nerves were first noted in the lower forelimbs at 30 months PI. E038 had LL leprosy 55 months PI which evolved histopathologically to BB leprosy 76 months PI, but again evolved to disseminated LL_s leprosy with extensive neural involvement by 83 months PI. E038 had a persistent IgM : IgG anti-PGL-I Ab ratio of <1 and failed to have detectable serum PGL-I Ag throughout the course of disease (Figure 5).

E040 (Figure 8), a second partially leprosy-resistant animal ('susceptible with resistance', see Table 1), showed an IgM : IgG anti-PGL-I Ab ratio $\ll$ during the early stages (10–18 months PI) prior to the progression of Ind dermal lesions at inoculation sites (Figure 8). After 20 months of incubation, skin lesions, with Ind leprosy classification, remained at inoculation sites. During the ensuing 10–12 months, the disease progressed together with the appearance of neuritic deformities in E040 and an increase in the anti-PGL-I IgM : IgG ratio to >1. Thereafter, clinical dissemination to the uninoculated tail was observed, but the overall condition of E040 remained definable as regressing until 40 months PI. By 40–46 months PI, the dermal disease again activated at some inoculation sites and at sites of dissemination on the tail. Histopathologically, the disease was classifiable as subpolar lepromatous leprosy (LL_s), and extreme neurologic deformities had appeared. By 51 months PI, AFB remained prevalent in the nasal secretions of E040 and dermal lesions remained at inoculation sites and on the tail. Histopathologically, the majority of the dermal disease at 51 months PI was located in the nerves as LL_s (Figure 8); simultaneously, Ind leprosy was observed in biopsies from other dermal lesions. Nerves in the distal forelimbs remained grossly enlarged. The dermal disease in E040 ceased to progress by 47–53 months PI, but the neurologic disease became severe, presumably causing the SMM to self-mutilate, necessitating treatment. During this static phase in dermal disease, the anti-PGL-I IgM : IgG ELISA ratio began to spontaneously fall to nearly 1, and continued to fall after clofazimine (CLFZ) was initiated at 51 months and RFM was added at 53 months PI. At the time of treatment, the preponderance of AFB, histopathologically, were located within dermal waves. E040 had a IgM : IgG anti-PGL-I ratio >1 coincidental with increasing leprosy dissemination and evolution to LL_s leprosy even though no serum PGL-I Ag was detectable and the disease stage never exceeded 2 (Figure 8).

Similarly, serum PGL-I Ag was absent in the other partially resistant SMM E038 (Figure 5) and all 4 of the resistant SMMs (Figures 2, 6, 7 and 10). The partially resistant SMMs, E038 and E040, had in common a strong tendency towards severe neural involvement and no detectable serum PGL-I Ag.

SMM E047 (Figure 9) received the same low dose of ML by the same route as E040 ($4{\cdot}5 \times 10^8$ IV and 4×10^6 IC), but followed a 'susceptible' course of disease similar to the high-dose SMMs E045, E043 and E044. In SMM E047, the ratio of anti-PGL-I IgM : IgG ELISA values was <1 in the early months PI until the disease began to

progress rapidly with the appearance of PGL-I Ag in the serum when the ratio began to exceed 1. At about 35 months PI, there was a temporary natural regression of clinical symptoms, a disappearance of PGL-I Ag from the serum and a simultaneous drop in the IgM : IgG ratio to < 1. Thereafter the clinical symptoms again began to progress, together with a reappearance of PGL-I Ag in the serum and an increase in the anti-PGL-I IgM : IgG ratio to >1 (Figure 9).

SMM E046 (Figure 11) received $4{\cdot}5 \times 10^8$ ML by only an IV route, developed LL leprosy, and followed a path similar to the other susceptible animals (E045, E044, E043 and E047). E041 (Figure 10), which received the same dose of $4{\cdot}5 \times 10^8$ ML by an IV only route (see above), was categorized as resistant (Table 1). E046 developed continuously progressive BL–LL leprosy by 24 months PI, together with continuously increasing serum PGL-I Ag levels and anti-PGL-I IgM : IgG ratio, both peaking at approximately 30–36 months PI. Thereafter, the dermal disease spontaneously regressed in E046, beginning at about 34 months PI, coincident with significant neural leprosy symptoms. The IgM and IgG anti-PGL-I Ab ratio and the PGL-I serum Ag level remained essentially constant, however, until RFM chemotherapy was initiated at 39 months PI. Chemotherapy resulted in falling IgM and IgG anti-PGL-I ELISA values (the ratio remained constant) and eventual curing of dermal leprosy.

From the foregoing results, 5 of 11 SMM (E045, E044, E043, E046 and E047) that were previously categorized as susceptible, based on clinical observations, were all found to have serologic indications in agreement with this designation, namely elevated proportions of anti-PGL-I IgM : IgG ELISA values and detectable PGL-I Ag levels in the circulation reflecting a high ML load,[1] and 4 additional SMM (D215, E039, E041 and E042) previously categorized clinically as resistant were found to lack detectable serum PGL-I Ag and often had very low or insignificant absolute levels of Ab. Where anti-PGL-I Ab levels were significant in those 4 resistant SMM, the IgM : IgG anti-PGL-I ratios remained nearly 1 or < 1 throughout.

The 2 SMM, E038 and E044, which had been categorized as being susceptible with resistance had more moderate dermal indications of leprosy than the susceptible SMMs (E038, no greater than 3+; E040, no greater than 2+), 1 or more episodes of pronounced spontaneous regression, more gradual onset of disease symptoms, severe neural involvement and they lacked detectable serum PGI = I Ag. The IgM : IgG anti-PGL-I Ab ratio remained < 1 in E038 for the duration of this study. E038 initially developed BB–BL leprosy, evolved after regressive episodes to BB and, ultimately, to LL_s. The < 1 anti-PGL-I IgM : IgG ratio is consistent with the immunologic resistance (more cell-mediated immunity (CMI) against ML) implied by the classification[6] closer to BB for most of the period of study in E038. In E040, which initially developed BL–LL leprosy ultimately evolving to predominantly neural LL_s leprosy (implying less CMI than E038), however, after 18 months PI the proportion of IgM : IgG became and remained $\gg 1$ until after chemotherapy initiation even though dermal disease severity never exceeded the 2+ stage (Figure 8). E040 represents an exception to our usual observations in that it was the only SMM with minimal dermal leprosy (albeit BL–LL or LL_s disease), a tendency towards resistance and predominantly neural LL_s leprosy, no detectable serum PGL-I Ag and, yet, it displayed a very large IgM : IgG anti-PGL-I ELISA Ab ratio for the majority of the study. Nevertheless, the high proportion of IgM : IgG anti-PGL-I is consistent with the disease classification near the LL end of the spectrum. E038 appeared clinically more resistant than E040 and the anti-PGL-I IgM : IgG proportion was consistent with that interpretation.

In all animals where PGL-I Ag was detectable, the peak(s) of Ag concentration coincided with peaks of clinical exacerbation of disease and periods during which the proportion of IgM anti-PGL-I Ab values increased to > 1 relative to IgG anti-PGL-I Ab values; SMM with detectable serum PGL-I Ag levels greater than 1+ had 3+ to 4+ levels of disease severity (Figures 1, 3, 4, 9 and 11).

Anti-LAM IgG levels were elevated near or above the mean + 2 SD cut-off point of 0·324 in the susceptible SMMs E045, E044 and E047 prior to inoculation, but were negligible in the susceptible SMMs E043 and E046 (Figures 1, 3, 4, 9 and 11). Among the 4 high-dose combined IV/IC route recipients, anti-LAM IgG Ab fluctuated with time but peaked together with serum Ag load in E045 and E044 as well as the low dose susceptible SMM E047 (Figures 1, 4 and 9).

Anti-LAM IgG was below the cut-off point prior to inoculation in the leprosy-resistant SMM_s E039, D215, E041 and E042 (Figures 2, 6, 7 and 10) and the 2 SMMs showing regressive episodes followed by continued progression and neuritic complications, i.e. the 'susceptible with resistance' animals E038 and E040 (Figures 5 and 8). The anti-LAM IgG level remained below the positive level throughout the study in the resistant SMMs E039, D215 and E041 but rose above the positive level in E038 and E040, the SMMs designated as 'susceptible with resistance', concurrently with LL leprosy appearance.

Anti-LAM IgM rose to a sustained peak considerably in excess of the cut-off point in the susceptible animals E043 and E046 in unison with peak clinical symptoms and PGL-I Ag loads (Figures 3 and 11) and in resistant SMM E041 (Figure 10); anti-LAM IgM levels exceeded the cut-off point only transiently or not at all in other susceptible (E046 and E047, Figures 9 and 11), susceptible with resistance (E038 and E040; Figures 5 and 8) and resistant (E039, E042 and D215; Figures 2, 6 and 7) SMM. Anti-LAM IgM levels peaked just above the cut-off points coincidently with peak PGL-I Ag levels in susceptible SMMs E045 and E044.

Elevated Ab levels and PGL-I Ag levels fell within 4–11 months after treatment, except for anti-LAM IgG levels which, once elevated, generally remained elevated throughout the period of study (Figures 3, 8, 9 and 11).

Discussion

The results extend and are in basic agreement with our prior observations in other groups of monkeys and in chimpanzees.[1–3] First, susceptibility to experimental leprosy was variable from animal to animal, including different SMMs inoculated with identical doses of ML via the same route(s). In all, 3 of 4 SMMs given the highest dose (approximately $10{\cdot}5 \times 10^8$ organisms) by combined IV/IC routes developed progressive, disseminated LL leprosy, whereas the 4th animal (E042) failed to develop clinically recognizable leprosy, as described in Part I.[9] An SMM (E038) given 6×10^8 ML + 4×10^6 IV ML and another (E040) given 6×10^6 IC + $4{\cdot}5 \times 10^8$ IC developed leprosy with regressive episodes ultimately culminating as LL_s leprosy, whereas another SMM (E047) given 6×10^6 IC + $4{\cdot}5 \times 10^8$ IV ML developed progressive, disseminated LL leprosy.[9] Of 2 SMM given 6×10^8 ML by only the IC route, 1 (D215) failed to develop disease and the other (E039) developed spontaneously regressive LL leprosy which self-healed.[9] Of 2 animals receiving $4{\cdot}5 \times 10^8$ ML by IV route only, 1 (E041) failed

to develop progressive disease and the other (E046) developed progressive BL–LL leprosy which required chemotherapy by 46 months PI.[9]

The described differences between individual SMM in clinical manifestations of experimental leprosy during early stages PI, a time in which it is not feasible to study leprosy in humans, clearly indicate that individuals differ from each other initially in the likelihood of developing leprosy after exposure to a given number of ML. Moreover, a given inoculum by a given route or routes can induce different classifications of disease in different SMMs. Based on these results, it is conceivable that a given individual may be resistant to clinical leprosy at a given ML dose level but may be susceptible at a higher ML dose.

The reasons for the observed individual differences in leprosy susceptibility are unknown but are almost certainly multifaceted and could include genetic differences, prior exposure to other mycobacteria and the presence of other unknown infectious agents.

As previously suggested, IgM anti-PGL-I ELISA OD values lower than IgG anti-PGL-I OD values were usually observed in animals that were resistant to LL leprosy (E042, E039 and D215) and/or during periods of resistance in SMM with spontaneously regressive episodes (E047) or prior to advancement/dissemination in SMM that developed LL leprosy more slowly (E044). Conversely, IgM anti-PGL-I OD values were higher than IgG A-PGL-I OD values in susceptible animals that rapidly developed LL forms of leprosy (E045 and E043) and/or during periods of dissemination and advancement in animals with LL leprosy (E043, E044, E046, E047 and E040). Thus, in leprosy-resistant animals and/or animals undergoing periods of spontaneous regression, the proportion of ELISA-determined IgM : IgG anti-PGL-I Ab OD values is generally < 1, whereas the IgM : IgG anti-PGL-I proportion is generally > 1 in leprosy-susceptible SMMs and/or during periods of advancement/dissemination of multibacillary leprosy. E040 is consistent with these observations in that the proportion of anti-PGL-I IgM exceeded the IgG throughout the course of leprosy (BL–LL) onset, progression, dissemination to the tail, regression and further progression to sustained, neural LL_s leprosy (Figure 5). Clinically, E040 initially appeared more like a susceptible animal in accord with the IgM : IgG ratio, since it developed BL–LL leprosy at inoculation sites and AFB could be found in nasal mucous (dissemination). E040 manifested resistant indications, however, such as regressive episodes, disease severity or stage no greater than level 2 +, no PGL-I Ag detectable in the serum and, ultimately, neural LL_s leprosy with some Ind leprosy lesions remaining simultaneously at dermal inoculation sites. This combination of observations clearly shows E040 to be an unusual susceptible case. E047, which received the same doses as E040 by the same combined IV/IC routes, was clearly a susceptible SMM.

Essentially all ML-inoculated SMMs had an initial ELISA spike of IgM A-PGL-I higher than IgG A-PGL-I within the first 6 months PI. The IgM : IgG Ab ratios later changed depending on whether regression or advancement of disease occurred. Thus, the ELISA results obtained in the first 5–6 months PI can be misleading and do not necessarily follow the generalizations. For this reason the ELISA data spanning approximately the first 6 months PI is not considered in this report. Aside from this initial spike, which soon subsided, SMMs that were resistant to leprosy or that developed early lesions at inoculation sites followed by spontaneous healing (E039, E041, D215 and E042) maintained anti-PGL-I IgM : IgG ratios < 1 or had very low

levels of Abs, often below the cut-off points, in which case the significance of the ratio is diminished and/or misleading.

In all cases of LL leprosy in which high levels of PGL-I Ag were found in sera, the ratio of ELISA-determined OD values of IgM : IgG anti-PGL-I was > 1. We have previously reported that advancing/disseminating LL leprosy in SMM coincides with a longitudinally-decreasing ratio of T-helper : T-suppressor cells in peripheral blood and with decreasing responses to the mitogens PHA, Con A and PWM,[7] and we have recently confirmed that observation in additional SMMs (B. Gormus *et al.*, manuscript in preparation). It, therefore, appears likely that variations in A-PGL-I Ab isotype levels directly or indirectly reflect changes taking place in lymphocyte subset regulation or involvement in cellular immunity during stages of progression or spontaneous regression of leprosy after ML infection.

As before, an association was observed between leprosy-susceptibility vs resistance and the initial IgG anti-LAM level,[2] and 3 of 4 SMMs with the greatest susceptibility to LL leprosy (E045, E044 and E047) had preinoculation anti-LAM IgG ELISA levels near or above the mean + 2 SD cut-off level of 0·32; 2 LL-leprosy-susceptible SMMs (E043 and E046) had an initial anti-LAM IgG level near zero; 5 of 6 of the more resistant SMMs (E038, E039, D215, E040 and E041) had anti-LAM IgG ELISA levels $\ll 0{\cdot}32$; and the remaining more resistant animal, E042, had an anti-LAM IgG level of 0·28, near but below the cut-off point. Thus, high ELISA-determined OD values for IgG antibody to the mycobacterial common, ubiquitous LAM Ag is associated with increased susceptibility to LL leprosy, in agreement with our prior results.[1] The mechanism of the increased susceptibility to multibacillary leprosy in the presence of high anti-LAM antibody levels is not known, but may be related to prior exposure to 'environmental' or to pathogenic mycobacteria, possibly by way of cross-reacting antigens or epitopes among mycobacteria.

Anti-LAM IgG appears to be the last of the 4 Abs studied longitudinally to diminish after treatment. As previously observed by Cho *et al.*,[5] PGL-I Ag disappears from serum within months post-treatment in LL leprosy accompanied by a decline in anti-PGL-I IgG and IgM towards baseline values.

Anti-LAM IgM was only transiently elevated above the 0·124 cut-off point in SMM E038 which was LL-susceptible, but which exhibited periods of resistance evidenced by spontaneous regression, and in SMM E039 and E040 which were relatively LL-leprosy resistant. Anti-LAM IgM was persistently elevated in E041, a leprosy-resistant animal which lacked serum PGL-1 Ag and in 3 LL leprosy susceptible SMMs, E045, E043 and E046, each of which had detectable serum PGL-1. Ag. Thus, 3 (E045, E043 and E046) of the 4 with persistent positive anti-LAM IgG ELISA levels each had detectable PGL-I Ag. These observations suggest that anti-LAM IgM appears and persists in the presence of high ML loads reflected by high serum PGL-I Ag levels. The exception, E041, had transient lesions on and near the scrotum, an uninoculated site, indicating that dissemination had occurred even though a high ML load was not present as reflected by elevated serum PGL-I Ag levels detected by dot-ELISA.

The observations confirm that longitudinal serum PGL-I Ag levels appear to correlate with higher ML loads and can be used to monitor the effectiveness of treatment.[5] Also, it could be predicted from our observations that longitudinal monitoring of IgG and IgM A-PGL-I Ab OD values by ELISA would be of importance in detecting leprosy contacts with pre- or subclinical leprosy.[1–3] Contacts with

persistently elevated anti-PGL-I IgM would be predicted to be highly at risk and those with persistently positive anti-PGL-I IgG OD values would be considered at a lesser risk of developing LL leprosy. The persistent longitudinal existence of positive ELISA OD values of anti-LAM IgG and/or IgM together with persisting or increasing positive anti-PGL-I IgM (and/or IgG) values would constitute the highest risk of incubating subclinical leprosy. Increasing IgM anti-PGL-I ELISA-OD values together with diminishing IgG anti-PGL-I OD values is an indication of an especially bad prognosis.[1,3]

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