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Serum Immunoglobulins, Including IgG Subclasses, in Vietnamese Leprosy Patients[1]

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Infection with *Mycobacterium leprae* leads, in a fraction of infected persons, to a long-term persistence of few or, in some cases, many bacteria. Even with treatment, bacteria may remain detectable for years. In this kind of chronic disease, some of the pathological effects are thought to be caused by an interference with normal immune regulation as a result of long-term interaction with persistent antigen(s). Although disturbances in cell-mediated immunity may be related to the inability of the host to cope with infection by *M. leprae* ([1, 14]), studies of the antibodies in leprosy patients can supply information about the state of the immune apparatus, since the proportions of different types of antibodies and the presence of antibodies not related to the specific immune response, such as autoantibodies, are indicative of the activities of the regulatory cells. In addition, since antibodies may also play a role in the regulation of immune responses and are an integral part of effector mechanisms, they may not only reflect the previous experience of the immune system but also its ability to react to future challenges.

Previous studies of humoral immunity in leprosy patients have demonstrated changes in patients compared to controls with respect to levels of IgG, IgM, IgA ([2, 3, 9, 21]) and IgE ([12]). Studies of antibodies to autoantigens in leprosy patients have given different results in different patient populations although, in general, the frequency of autoantibodies has been found to be increased in lepromatous patients ([11, 13, 15, 23]). Thus, there is increasing evidence from studies on immunoglobulins as well as on cells (reviewed by Rea [14]) that patients with lepromatous leprosy demonstrate abnormal B cell functions, probably reflecting abnormal T cell regulation.

We have begun a study of immunological factors in a group of patients from a leprosy center in the north of Vietnam. We report here the measurements of immunoglobulin levels in serum samples from patients with tuberculoid or lepromatous leprosy and from local controls, considering not only immunoglobulins G, M, A and E but also the subclasses of IgG. We have also screened a large number of both controls and patients for the presence of autoantibodies.

MATERIALS AND METHODS

Subjects. Serum was collected from healthy donors in the blood bank of St. Paul's Hospital in Hanoi, Vietnam, for the local controls. The age and sex distributions of these people were the same as in the patient group. The patients were all from the Quynh Lap Leprosy Center in the north of Vietnam. They had been classified clinically according to the Ridley-Jopling scale ([16]); the tuberculoid end of the spectrum is here represented only by borderline tuberculoid (BT) patients. The lepromatous (LL) group included active and inactive patients. All of them were under treatment with dapsone (DDS) since diagnosis; the time since diagnosis ranged from 1–14 years (mean 6 years). The sera were kept frozen until tested.

Immunoglobulin measurements. For these measurements, 15 individuals from each of four groups were investigated: Netherlands controls, Vietnamese controls, BT patients, and LL patients. Immunoturbidometric determinations of IgG, IgA, and IgM were performed with the Vitatron PA 800 analyzer.

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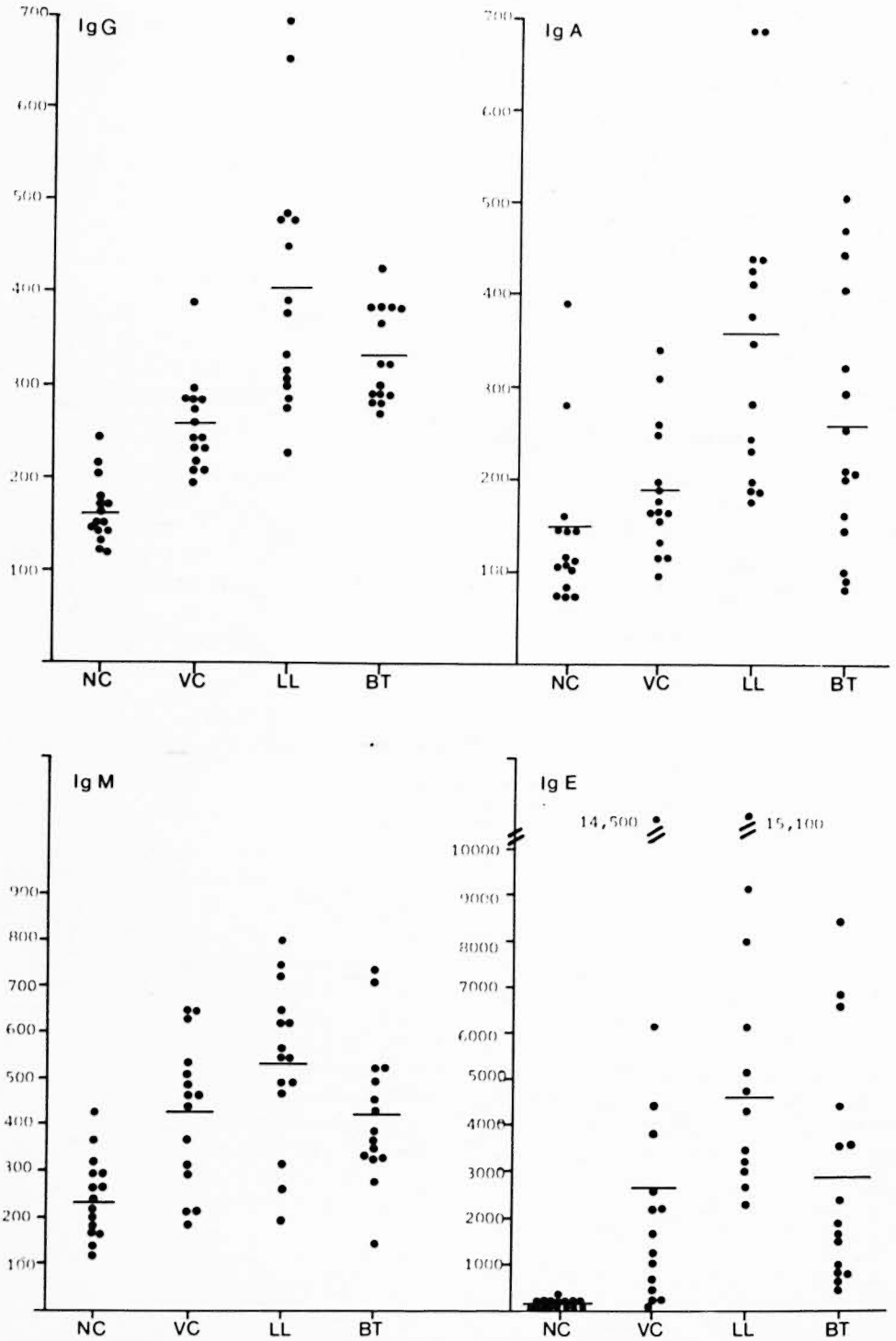

FIG. 1. Serum IgG, IgA, IgM, and IgE levels in Netherlands and Vietnamese controls and in Vietnamese leprosy patients. Immunoglobulin levels are expressed in international units per ml. NC = healthy Netherlands controls, VC = healthy Vietnamese controls, LL = lepromatous leprosy patients, BT = tuberculoid leprosy patients. Horizontal bars represent the mean in each group.

TABLE 1. *Immunoglobulin levels in sera of leprosy patients and controls.*

Immunoglobulins	Group (N = 15 in each)			
	NC[a]	VC[b]	LL	BT
IgG[c,d]	162 ± 35 (1303)	256 ± 47 (2058)	402 ± 138 (3232)	331 ± 51 (2661)
IgG_1[e]	108 ± 29	162 ± 35	291 ± 123	224 ± 57
IgG_2[e]	118 ± 56	182 ± 81	228 ± 86	167 ± 72
IgG_3[e]	107 ± 50	263 ± 84	409 ± 183	318 ± 101
IgG_4[e]	129 ± 82	221 ± 325	548 ± 384	480 ± 272
IgA[c,d]	148 ± 90 (216)	187 ± 71 (266)	361 ± 164 (513)	260 ± 142 (369)
IgM[c,d]	239 ± 86 (203)	427 ± 157 (373)	534 ± 174 (454)	425 ± 155 (361)
IgE[e]	94 ± 113	2771 ± 3681	4568 ± 3869	2981 ± 2549

[a] NC = Netherlands control group.
[b] VC = Vietnamese control group.
[c] I.U./ml, mean ± standard deviation.
[d] (mg/100 ml), mean, using conversion factors of 0.0804 for IgG, 0.0142 for IgA, and 0.00847 for IgM for the WHO standards.
[e] Percent of a standard Netherlands control serum.
Statistical analysis (Mann-Whitney U test):
1) Netherlands controls vs Vietnamese controls, significant differences in IgG ($p < 0.001$), IgA ($p < 0.025$), IgM ($p < 0.001$), IgE ($p < 0.001$), IgG_1 ($p < 0.001$), IgG_2 ($p < 0.01$), and IgG_3 ($p < 0.001$).
2) Vietnamese controls vs LL patients, significant differences in IgG ($p < 0.001$), IgA ($p < 0.001$), IgM ($p < 0.05$), IgE ($p < 0.05$), IgG_1 ($p < 0.001$), IgG_3 ($p < 0.025$), and IgG_4 ($p < 0.001$).
3) Vietnamese controls vs BT patients, significant differences in IgG ($p < 0.001$), IgG_1 ($p < 0.01$), IgG_3 ($p < 0.05$), and IgG_4 ($p < 0.05$).
4) LL patients vs BT patients, significant differences in IgM ($p < 0.05$).

The IgE levels were measured in a radioimmunoassay (kindly carried out by Dr. R. C. Aalberse, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service [CLB]). The IgG subclass levels were determined by radial diffusion using subclass-specific antisera ([5, 22]) obtained from the CLB. The differences in the immunoglobulin levels were evaluated statistically with the Mann-Whitney *U* test.

Autoantibody assays. For the autoantibody survey, more sera were tested: 100 Vietnamese controls and 91 leprosy patients (58 LL, 4 BB/BL, 29 BT), with the time under DDS treatment ranging from 1–30 years. In addition to the leprosy patients, a small group of tuberculosis patients was included for comparison. The Netherlands control sera results were selected from a large number of controls to give an age and sex distribution comparable to the Vietnamese sera.

The sera were tested for the following antibodies by immunofluorescence (using an FITC-labeled sheep anti-human immunoglobulin serum from the CLB): anti-nuclear antibody (ANA), serum 1/10, rat liver substrate; anti-parietal cell antibody (Par.c.ab.), serum 1/20, rat stomach substrate; anti-smooth muscle antibody (SMA), serum 1/20, rat stomach substrate; anti-mitochondrial antibody (AMA), serum 1/20, rat kidney substrate; anti-reticulin antibody (Ret Ab), serum 1/20, rat stomach and kidney substrate. The IgM rheumatoid factor (IgM RF) was measured in an ELISA at a serum dilution of 1/100.

RESULTS

The serum immunoglobulin levels of the controls and the patients are shown in Figures 1 and 2. A dramatic difference can be seen in the amounts of IgG: the level of IgG is higher in the Vietnamese controls than in the Netherlands controls ($p < 0.001$); it is also significantly higher in both the tuberculoid ($p < 0.001$) and the lepromatous ($p < 0.001$) patient groups than in the local controls (Table 1). There was no significant difference between the LL and BT patient groups. There were similar but less striking differences in the IgM levels. IgA was slightly increased in the Vietnamese controls compared with the Netherlands controls ($p < 0.025$) and in the BT patients, while it was significantly higher in the LL patient

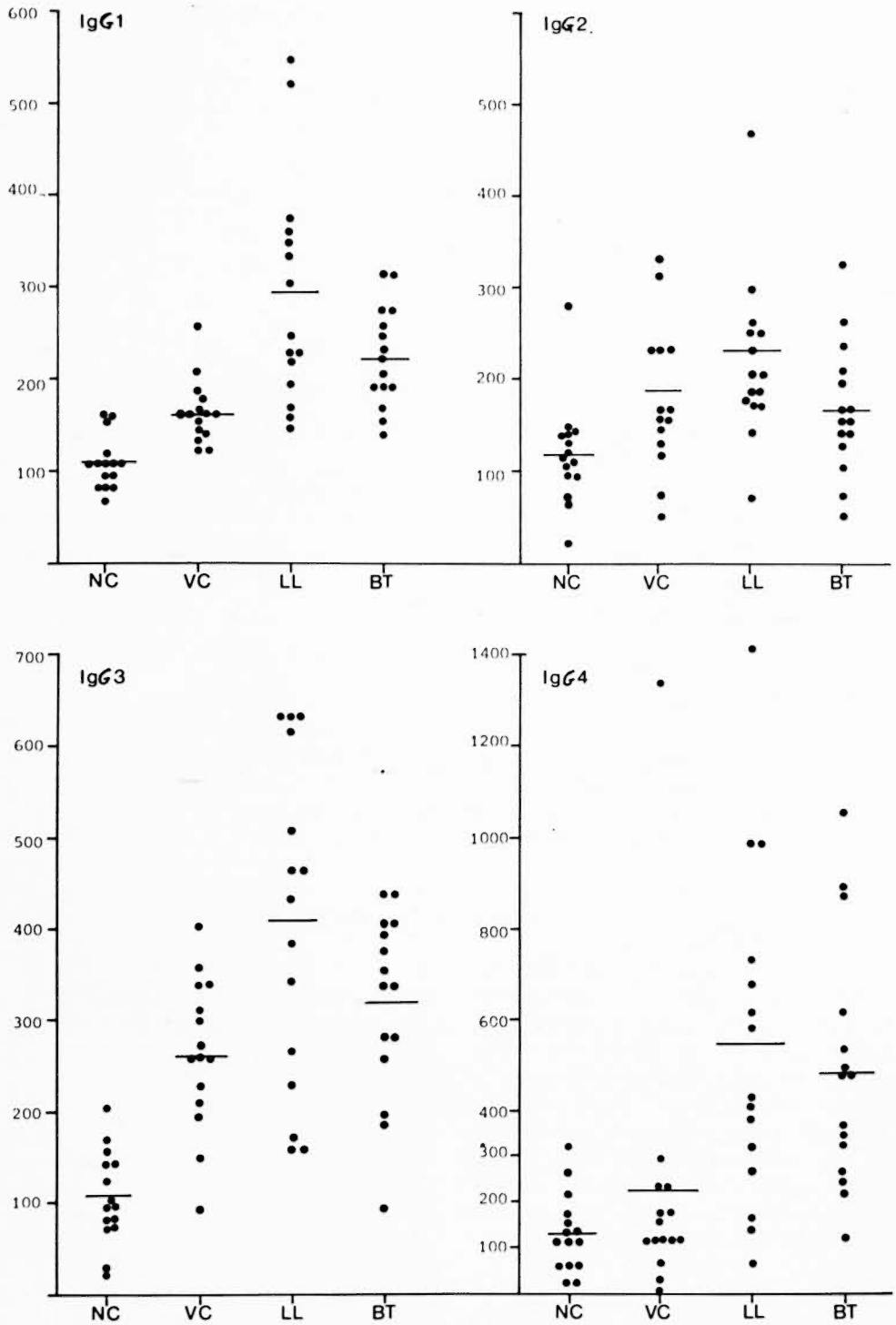

FIG. 2. Serum levels of IgG subclasses in Netherlands and Vietnamese controls and in Vietnamese leprosy patients. Units of immunoglobulins are related to a standard reference serum (a Dutch human serum with 6.2 mg/ml IgG_1, 2.4 mg/ml IgG_2, 0.64 mg/ml IgG_3, and 0.46 mg/ml IgG_4). (See Figure 1 for abbreviations.)

TABLE 2. *Autoantibodies in Vietnamese leprosy patients and in controls.*

Group	No.	Antibodies[a]					
		ANA	Par cAb	SMA	AMA	Ret Ab	IgM RF
Vietnamese controls	100	3[b]	1	7	0	2	20
Leprosy patients							
BT	29	0	0	1	0	0	6
LL	58	1	0	1	0	1	15
BB/BL	4	0	0	0	0	0	2
Total	91	1	0	2	0	1	23
Tuberculosis patients	15	1	0	1	0	0	2
Netherlands controls	100	1	2	2	0	3	5

[a] See Materials and Methods section for antibody abbreviations.
[b] Number of positive sera.

group than in the local controls ($p < 0.001$). IgE was very low in the Netherlands control group and significantly higher in the Vietnamese control group ($p < 0.001$). The LL, but not the BT, patient group had IgE levels significantly higher than the local controls ($p < 0.05$) but there was great variation within each of these groups.

Figure 2 shows the amounts of the four subclasses of IgG in the sera from the controls and the patients. The Vietnamese controls differed significantly from the Dutch controls in the amounts of IgG_1, IgG_2, and IgG_3, but not in IgG_4. Leprosy patients had significantly more IgG_1 and IgG_4 than did the local controls and slightly (but significantly) more IgG_3. Although the mean IgG_2 was raised in the patient groups, the difference was not significant. There were no significant differences between the LL and BT patient groups with respect to the amounts of the subclasses of IgG. Since the total IgG in the patient groups was considerably higher than in the controls, it was to be expected that the subclasses would also be increased. The differences were not, however, equally great for each of the four subclasses. Table 1 summarizes the data together with the results of the statistical tests.

The results of the screening for autoantibodies are shown in Table 2. It can be seen that the Vietnamese controls, although they had high amounts of immunoglobulins as shown above, did not have a high frequency of autoantibodies. The corresponding values for the healthy Dutch population were essentially the same as for the Vietnamese with the exception of the IgM RF. Further, neither of the patient groups exhibited an increase in autoantibodies over the controls from the same area. The patients also had a high frequency of IgM RF, but not higher than the local controls. The tuberculosis patients were similarly lacking in autoantibodies.

DISCUSSION

In developing countries, the variety and quantity of antigenic stimulation results in an immunological profile somewhat different from that seen in industrialized countries. These changes include elevation of serum levels of IgG, IgM, IgA, and some autoantibodies ([6]). In the present study, we have found a considerable increase in IgG in the Vietnamese controls compared to the controls from the Netherlands. IgA and IgM were also elevated, although less so, and IgE was greatly increased. The latter may be related to the ubiquitousness of helminth infections in Vietnam. When we looked at the subclasses of IgG, the Vietnamese controls had increased amounts of all but IgG_4 compared to the Dutch controls.

Leprosy patients have been reported to have increased immunoglobulin levels when compared to local controls. Bullock, *et al.* ([2]) reported increases in IgG and IgA but not IgM in patients at the lepromatous pole. Increases were also reported in lepromatous leprosy by Turk and Bryceson ([21]). In a series of studies in India (summarized by Kelkar, *et al.*[9]) IgG levels were usually increased in both LL and TT patients, while IgM and IgA were increased only in LL patients, although there was some variation among these studies. Later studies in India ([7, 10]) demonstrated increased IgG, IgM, and IgA

in lepromatous but not in borderline or tuberculoid leprosy patients. The patients in these different studies not only came from different population groups but undoubtedly differed as to clinical conditions and length of time and nature of treatment, which might account for some of the variations in the results reported. In this study, the lepromatous leprosy patients, all of whom had been under DDS treatment for some time, had significant increases in serum levels of IgG, IgA, and IgM in comparison with local controls, while the BT patients differed from local controls only by an increase in IgG. Nuti, *et al.* [12] found both TT and LL patients to have significantly increased IgE levels, and their LL patients had significantly more IgE than did their TT patients. In the groups under study here, only the LL patient group had significantly more IgE than the local control groups, while all of the Vietnamese had more IgE than did controls from the Netherlands. There was also considerable variation within groups from Vietnam.

When the IgG subclasses were examined, the LL patients differed from the controls in IgG_1, IgG_3, and IgG_4 but not in IgG_2, and the same difference was found in the BT patients. Shifts in the IgG subclass distribution have been noted in other diseases. Salimonu, *et al.* [18] reported increased IgG_1 in high-serum-IgG malaria patients, while their IgG_3 was slightly decreased. Marked elevation of IgG_3 and IgG_4 was observed in schistosomiasis patients, besides the expected high IgE, by Iskander, *et al.* [8]. Hodgkin's disease patients treated with radiation and chemotherapy had, besides decreased IgA and increased IgM, a decrease in IgG_2 (with normal total IgG) accompanied by a reduced antibody-forming response to bacterial polysaccharides [20].

During normal maturation of the immune response in young people, the amounts of IgG_2 and IgG_4 rise slowly with age, while IgG_1 and IgG_3 reach adult levels very early [22]. The expression of different IgG subclasses, like that of different classes of immunoglobulins, reflects different activities of regulatory factors from within the system or outside it. Thus the changes seen, especially in the LL patients, are evidence of disturbed immune regulation. Since some antigens are known to induce preferentially one or another subclass of antibody [17], it would be interesting to test the *M. leprae*-specific antibodies as to subclass distribution.

A contribution of long-term treatment with DDS to the observed alterations in serum immunoglobulins cannot be excluded. Not only might DDS influence the expression of antibodies (although the less-marked differences between the DDS-treated BT patients and the controls argues against a strong influence), but some of the antibodies might be directed against the drug itself [4].

Autoantibodies are reported to be increased in developing countries, perhaps as a result of frequent viral or protozoal infection [6]. However, in the Vietnamese controls studied here, only IgM rheumatoid factor was found more frequently than in the Dutch population. Similarly, several studies have reported an increased frequency of autoantibodies in leprosy patients, especially at the lepromatous pole, and cited this as evidence for disturbed immune regulation [11, 13, 15, 19, 23]. In the patients studied here, however, the frequency of autoantibodies to tissue antigens was very low and equally distributed among different patient groups. The frequency of IgM RF in the leprosy patients was high compared to the Netherlands controls, but since this was also true for the healthy Vietnamese controls, it is apparently related to the environment and not to the disease.

In this study, therefore, we have shown that Vietnamese lepromatous leprosy patients had significantly raised levels of serum IgG, IgA, IgM, and IgE, while BT patients had only increased IgG in comparison to local controls. The IgG_2 subclass was not increased in the patients but the other subclasses of IgG were. There was no evidence of extensive autoantibody formation. Thus, although some disturbances of immune regulation were observed, this did not involve all aspects of antibody formation but did involve increased levels of immunoglobulins. It will be of interest to investigate whether the antibodies specific for the infection show some of the same differences as the immunoglobulins as a whole.

SUMMARY

Levels of serum immunoglobulins were measured in healthy Vietnamese and in lep-

rosy patients. Healthy Vietnamese had higher levels of IgG, IgA, IgM, and IgE than did healthy Dutch controls, as well as higher levels of three of the four subclasses of IgG (IgG_1, IgG_2, IgG_3). Lepromatous leprosy patients had significant increases in all classes and subclasses of immunoglobulins, except for IgG_2, in comparison with local controls. Tuberculoid leprosy patients had more IgG_1, IgG_3, and IgG_4 than did local controls and had higher total IgG levels. The patients had no increase in autoantibodies against tissue antigens compared to local or Dutch controls.

RESUMEN

Se midieron los niveles de las inmunoglobulinas séricas en vietnamitas sanos y en pacientes con lepra. Vos vietnamitas sanos tuvieron niveles más altos de IgG, IgA, IgM e IgE, que un grupo de controles holandeses, así como niveles más altos de tres de las cuatro subclases de IgG (IgG_1, IgG_2 e IgG_3). Comparados con los controles locales, los pacientes con lepra lepromatosa tuvieron incrementos significantes en todas las clases y subclases de las inmunoglobulinas, excepto en IgG_2. Los pacientes con lepra tuberculoide tuvieron más IgG_1, IgG_3 e IgG_4 que los controles locales, así como niveles más elevados de IgG total. Los niveles de autoanticuerpos contra antígenos tisulares fueron similares en los pacientes y en los controles locales o en los holandeses.

RÉSUMÉ

On a procédé à la détermination des taux d'immunoglobulines sériques chez des vietnamiens en bonne santé et chez des malades de la lèpre. Les vietnamiens en bonne santé présentaient des taux plus élevés d'IgA, d'IgM et IgE que les témoins hollandais en bonne santé. Ils présentaient également des taux plus élevés pour trois des sous-groupes d'IgG, à savoir IgG_1, IgG_2, et IgG_3. Les malades atteints de lèpre lépromateuse témoignaient d'une augmentation significative de tous les groupes et sous-groupes d'immunoglobulines, à l'exception des IgG_2, par comparaison avec les témoins locaux. Les malades souffrant de lèpre tuberculoïde avaient des taux plus élevés d'IgG_1, IgG_3, et d'IgG_4 que les témoins locaux; ils présentaient également des taux plus élevés d'IgG totales. Aucune augmentation des auto-anticorps contre les antigènes tissulaires n'a été observée chez les malades, par rapport aux témoins locaux ou aux témoins hollandais.

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