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Serum Immunoglobulins and Autoantibodies During and After Erythema Nodosum Leprosum (ENL)[1]

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Erythema nodosum leprosum (ENL) is a serious complication seen in highly bacilliferous leprosy patients ([4, 10]). Its immunopathogenesis, based on circulating and tissue deposited immune complexes, has been amply documented ([1, 19]). An increased prevalence of organ-specific as well as non-organ-specific autoantibodies has been shown during ENL reactions in lepromatous leprosy, although an immunological profile of serum immunoglobulins and autoantibodies after the subsidence of the ENL has not been adequately studied ([6, 7, 13, 17]). The present report deals with studies of the above parameters over the course of four weeks, during and after ENL, during which time the patients were under our direct supervision.

MATERIALS AND METHODS

Twenty patients with ENL were studied. These patients were from the Urban Leprosy Centre, National Leprosy Control Programme, Safdarjang Hospital. Their mean age was 36.2 years, with a range of 22 to 52 years. The primary diagnosis was established in each case according to the criteria of Ridley and Jopling ([12]). Eight of the 20 patients were classified as borderline lepromatous (BL) and 12 as lepromatous (LL) leprosy. The Bacteriological Index of the patients ranged from 2+ to 4+. The duration of illness varied from 2 to 18 years, with a mean of 4.5 years. The diagnosis of erythema nodosum leprosum (ENL) was made clinically ([15, 16]). In nine patients the ENL was recorded for the first time; while in the rest of the patients there had been episodes of recurrence and remission. Each patient was treated with recommended drugs ([16]). Eleven patients received prednisolone, 20 mg daily for one week, followed by 5 mg daily. Three patients were treated with clofazimine (Lamprene®), 100 mg three times daily. The remaining six patients received chloroquin 250 mg three times daily. The duration of the anti-ENL therapy was four weeks in all but two cases who showed only partial relief with the prednisolone (one case) and chloroquin (one case) and continued to receive the drugs for six weeks.

Two samples of 5 ml of venous blood were collected from each patient. The initial sample was collected on the first visit, and the subsequent sample was drawn after complete remission of the ENL on the 28th–30th day of treatment. Sera were separated and stored in small aliquots at −20°C.

Immunoglobulins M, G, and A were measured in the paired sera from each patient by the single radial immunodiffusion technique ([8]) using monospecific anti-immunoglobulin antisera and reference standards obtained commercially (Meloy Laboratories, Springfield, Virginia, U.S.A.). Antinuclear antibodies (ANA) were demonstrated by the standard immunofluorescence technique ([20]) employing human buffy coat as substrate and fluorescein isothiocyanate (FITC) conjugated anti-human globulin antiserum (Meloy Laboratories, Springfield, Virginia, U.S.A.).

Antithyroglobulin antibody (ATA) against colloid thyroglobulin antigen was detected with the latex agglutination test using the thyroglobulin agglutino test kit (Immunitalia, Pomezia, Rome, Italy). Rheumatoid factor (RF) was detected by the latex agglutination technique using a Rheumatoid Factor Latex test kit (Laboratory Diagnostics Co., Morganville, New Jersey, U.S.A.). The

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TABLE 1. *Serum immunoglobulin levels in the sera of 20 patients during erythema nodosum leprosum (ENL) and following clinical remission. Values are given in mean mg/dl ± S.D. (range).*

Immunoglobulin class	During ENL	After clinical remission
M	170 ± 46 (90–275)	183 ± 49 (95–275)
G	1561 ± 260 (1025–1975)	1751 ± 292[a] (1125–2225)
A	121 ± 32 (50–195)	112 ± 28 (50–167.5)

[a] Significantly increased compared to acute ENL, $p < 0.05$, paired t test.

paired t test was used for calculations of statistical significance.

RESULTS

The serum immunoglobulin concentrations during and after erythema nodosum leprosum (ENL) are given in Table 1. There is a rise of 20% in the level of immunoglobulin G after clinical remission of ENL, which is statistically significant ($p < 0.05$). Immunoglobulin A levels tended to fall; while immunoglobulin M showed a tendency to rise, but these changes are not statistically significant. On further analysis of the data on the basis of the drug used as therapy for the ENL (Table 2, The Figure), there was a substantial increase in IgG levels in patients treated with prednisolone. Changes in IgG in the sera of those patients treated with either clofazimine or chloroquin were less marked. There was no significant alteration in either serum IgM or serum IgA. An increase in autoantibodies in the sera of these patients after remission of ENL (Table 3) was another interesting finding. The patients with autoantibodies tended to have a higher mean age (37.2 years) and a longer mean duration of illness (7.5) than those without autoantibodies. Furthermore, autoantibodies were predominantly detected in patients having repeated episodes of ENL. One of the patients had all the three autoantibodies in sera collected during and after the reaction. This patient had a recurrence of ENL thrice and had had the disease for 12 years. Among the cases treated with prednisolone, two patients who did not have ATA or ANA in their first samples developed these autoantibodies in the second samples collected four weeks later. One patient on chloroquin therapy also developed ATA in the second serum sample (Table 4).

DISCUSSION

Since the original description of ENL syndrome, its etiopathogenesis remains speculative. Various precipitating factors have been identified ([5, 15, 16]). Many workers

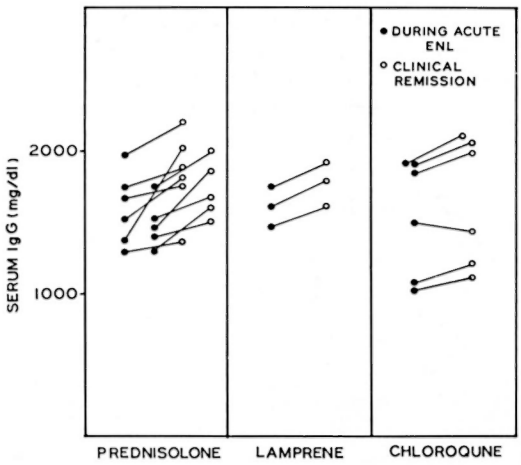
TABLE 2. *Effect of therapy on the serum immunoglobulin levels in 20 patients with erythema nodosum leprosum. Values are given in mean mg/dl ± S.D. (range).*

Drug	Patients	Time of serum collection	Immunoglobulin class		
			M	G	A
Prednisolone	11	During acute ENL	157 ± 35 (95–235)	1552 ± 199 (1300–1975)	116 ± 29 (50–157.5)
		Clinical remission	175 ± 50 (105–275)	1798 ± 230[a] (1375–2225)	108 ± 27 (50–127.5)
Chloroquin	6	During acute ENL	186 ± 34 (130–235)	1550 ± 381 (1025–1925)	133 ± 40 (70–195)
		Clinical remission	193 ± 44 (140–235)	1650 ± 384 (1125–2075)	126 ± 29 (75–167.5)
Clofazimine	3	During acute ENL	188 ± 80 (90–275)	1616 ± 112 (1475–1705)	104 ± 13 (85–115)
		Clinical remission	183 ± 62 (95–235)	1783 ± 123 (1625–1925)	97 ± 11 (82.5–110)

[a] Significantly increased compared to acute ENL, $p < 0.05$, paired t test.

TABLE 3. *Autoantibodies in the sera of 20 patients during erythema nodosum leprosum and following clinical remission.*

Autoantibodies	Number (%) of sera showing autoantibody	
	During acute ENL	Clinical remission
Antithyroglobulin antibody	4 (20)	7 (35)
Antinuclear antibody	4 (20)	6 (30)
Rheumatoid factor	1 (5)	1 (5)



THE FIGURE. Concentrations of immunoglobulin G in the paired sera of 20 ENL patients treated with different anti-ENL drugs. Lamprene® is clofazimine.

([6, 7, 13, 17, 18]) have studied the serum level of immunoglobulins in leprosy patients. These studies have largely reported increased levels of IgG in lepromatous leprosy patients, during both reactional as well as non-reactional phases of the disease process. Similarly, Reichlin, *et al.* ([11]) found lower levels of euglobulin IgG in LL patients without ENL compared to those with ENL. Bullock and co-workers ([3]) found that IgA, IgG, and IgM all were significantly increased in the sera of patients with lepromatous leprosy as compared with patients with tuberculoid leprosy and control subjects of the same racial and geographic group. The present study also has shown higher levels of IgG in patients with ENL, as compared with the levels in healthy controls and in leprosy patients estimated in this laboratory in an earlier study ([13]). A significant elevation of IgG was also observed in the sera after the ENL had subsided. This observation may signify that either there was increased synthesis of this immunoglobulin or that it was no longer being consumed in the formation of circulating and/or tissue deposited immune complexes. This increase in concentration was more evident in the prednisolone treated group than in patients treated with chloroquin or clofazimine. This may be due to the well known immunosuppressive action of prednisolone in contrast to the other drugs.

The occurrence of organ as well as non-organ-specific autoantibodies has been demonstrated in earlier studies ([4, 9, 13, 17]), though their prevalence is quite variable. In the present study, one patient showed persistent RF in the serum, both during and

TABLE 4. *Comparison of anti-ENL drugs in terms of clinical relief and immunological perturbation in 20 patients.*

Drug	Patients	Clinical remission		Immunologic perturbation in sera after clinical remission	
		In weeks	Number	Increase in serum IgG(%)	Appearance of autoantibody[a]
Prednisolone	11	1st 2nd	6 5[b]	16.0	2
Chloroquin	6	1st 2nd	3 3[b]	6.5	1
Clofazimine	3	1st	3	10.0	0

[a] Number denotes patients showing presence of autoantibody in previously negative sera collected after clinical remission.

[b] One patient in each group showed only partial relief clinically.

after ENL reaction. On the other hand, Saha and Mittal [13] and Sen Gupta, *et al.* [17] reported a positive RF factor in 35% and 25% of lepromatous leprosy patients, respectively. The presence of ANA has been similarly reported to vary from 0% to 30% [1], and the present observation of 20% and 30% in paired sera agrees closely with earlier data from this laboratory [14]. Similarly, autoantibodies to thyroglobulin have been reported by many workers [2, 4, 9, 14], the prevalence ranging from 5% to 20%.

Our study revealed some interesting findings. Autoantibodies were found in those patients whose mean age and duration of the disease were higher than other patients, and also in those cases who had episodes of ENL in the past. Furthermore, these autoantibodies (ATA and ANA) at times appeared for the first time during the reactional phase, and they appeared in the sera of some of those patients who had received prednisolone to control the ENL. This remarkable immunological perturbation during the course of ENL reaction, even after its clinical remission, suggests that there is a continuous deterioration of the immunological status of these patients during the prolonged and chronic course of the disease, associated with repeated attacks of ENL.

SUMMARY

Sera from 20 patients with lepromatous leprosy complicated by erythema nodosum leprosum (ENL) were collected at the time of acute reaction and then after clinical cure four weeks later. Anti-ENL drugs used were: prednisolone (11 patients), chloroquin (6 patients), and clofazimine (3 patients). Immunoglobulins M, G, and A and autoantibodies, namely, antithyroglobulin antibody (ATA), antinuclear antibody (ANA), and rheumatoid factor (RF), were measured in these 20 paired serum samples. The mean serum concentration of IgG showed a significant elevation after clinical subsidence of the reaction, mainly in the prednisolone treated group; while those of IgM and IgA varied only marginally. Autoantibodies were detected in nine patients. Of these, three patients developed these antibodies only after remission of the reaction. Treatment with prednisolone and chloroquin, although causing subsidence of ENL, resulted in an increased incidence of ATA and/or ANA. Furthermore, it was observed that longer duration of illness, higher age group, and history of repeated attacks of ENL predisposed these patients to enhanced autoantibody formation.

RESUMEN

Se colectaron los sueros de 20 pacientes con lepra lepromatosa complicada con eritema nodoso leproso (ENL) durante la fase aguda de la reacción y 4 semanas después, cuando se observó su curación clínica. Las drogas anti-ENL usadas fueron la prednisolona (11 pacientes), la cloroquina (6 pacientes), y la clofazimina (3 pacientes). En las 20 muestras pareadas de suero se midieron los niveles de las inmunoglobulinas M, G y A; de los anticuerpos antitiroglobulina (AAT), antinucleares (AAN), y de factor reumatoide (FR). La concentración media de IgG en el suero mostró una elevación significante después de la mejoría clínica del paciente, principalmente en el grupo tratado con prednisolona, mientras que los niveles de IgM y de IgA sólo variaron marginalmente. En nueve pacientes se encontraron autoanticuerpos. De éstos, tres desarrollaron los anticuerpos sólo después de ocurrir la remisión clínica de la reacción. El tratamiento con prednisolona y cloroquina, aunque ayudó a la remisión del ENL, favoreció la aparición de AAT y/o AAN. Se observó además, que la larga duración de la enfermedad, la edad avanzada de los pacientes, y la historia de ataques repetidos de ENL, predispusieron a los pacientes al desarrollo de una mayor incidencia de autoanticuerpos.

RÉSUMÉ

Chez 20 malades souffrant de lèpre lépromateuse, compliquée par un érythème noueux lépreux (ENL), on a recueilli du sérum au moment de la réaction aigue, et une seconde fois après la guérison, quatre semaines plus tard. Les médicaments utilisés pour maîtriser l'ENL étaient la prednisolone (11 malades), la chloroquine (6 malades), et la clofazimine (3 malades). Chez ces 20 échantillons appariés de sérum, on a mesuré les taux d'immuno-globuline M, G, et A, de même que les autoanticorps, en particulier, l'anticorps anti-thyroglubuline (ATA), l'anticorps anti-nucléaire (ANA), et le facteur rhumatoïde (RF). La concentration moyenne d'IgG dans le sérum présentait une augmentation significative après la disparition clinique de la réaction, surtout dans le groupe traité à la prednisolone, alors que les variations des taux d'IgM et d'IgA étaient liminaires. On a détecté des auto-anticorps chez 9 malades. Parmi ceux-ci, trois n'ont développé ces auto-anticorps qu'après la disparition de la réaction. Le traitement par la prednisolone et par la cloroquine, malgré qu'ils entraînent une disparition de l'érythème noueux lépreux, produisent une incidence accrue d'ATA et/ou d'ANA. De plus, on a observé que la

formation d'autant d'autocorps était renforcée par la durée de la maladie, l'âge, et des antécédents d'attaques répétées d'érythème noueux lépreux.

REFERENCES

1. Bjorvatn, B., Barnetson, F. S., Kronvall, G. K., Zubler, R. H. and Lambert, P. H. Immune complexes and complement hypercatabolism in patients with leprosy. Clin. Exp. Immunol. **26** (1976) 388–396.
2. Bonomo, L. and Dammano, F. Characterization of thyroglobulin antibodies in leprosy: An immunological study of diethyl amino-ethyl cellulose chromatographic fraction. Immunol. **13** (1967) 565–576.
3. Bullock, W. E., Jr., Min-Fu Ho and Mei-Jan Chen. Studies of immune mechanisms in leprosy. II. Quantitative relationship of IgG, IgA and IgM immunoglobulins. J. Lab. Clin. Med. **75** (1970) 863–870.
4. Bullock, W. E. Leprosy: A model of immunological perturbation in chronic infection. J. Inf. Dis. **137** (1978) 341–354.
5. Dutta, R. K. A study of patients with erythema nodosum leprosum syndrome. Lepr. India **51** (1979) 209–212.
6. Jha, P., Balkrishnan, K., Talwar, G. P. and Bhutani, L. K. Status of humoral immune response in leprosy. Int. J. Lepr. **39** (1971) 14–19.
7. Kelkar, S. S., Mondkar, A. D. and Warawadekar, W. Serum immunoglobulins in leprosy. Lepr. India **51** (1979) 189–193.
8. Mancini, G., Carbonara, A. C. and Heremans, J. P. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry **2** (1965) 235–254.
9. Masala, C., Amendolea, M. A., Nuti, M., Riccarducci, R., Tarabini, C. G. L. and Tarabini, C. G. Autoantibodies in leprosy. Int. J. Lepr. **47** (1979) 171–175.
10. Rea, T. H. and Levan, N. E. Current concepts in the immunology of leprosy. Arch. Derm. **113** (1977) 345–352.
11. Reichlin, M., Pranis, R. A., Gelber, R. H., Rees, R. F., Taverne, F. and Turk, J. L. Correlation of euglobulin immunoglobulin G levels with erythema nodosum leprosum in lepromatous leprosy. Clin. Immunol. Immunopathol. **8** (1977) 335–344.
12. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity. A five group system. Int. J. Lepr. **34** (1966) 255–273.
13. Saha, K. and Mittal, M. M. Immunological aspects of lepromatous leprosy with special reference to the study of antibodies. Int. J. Lepr. **40** (1972) 260–264.
14. Saha, K., Dutta, R. N. and Mittal, M. M. Immunologic aspects of leprosy as related to leucocytic isoantibodies and platelet aggregating factors. Int. J. Lepr. **43** (1975) 239–248.
15. Sehgal, V. N. *Clinical Leprosy*. 1st ed. Sahibadad, Distt. Ghaziabad, India: Vikas Publishing House Pvt. Ltd., 1979, pp. 52–58, p. 197.
16. Sehgal, V. N., Rege, V. L. and Mascrarrnhas, M. F. Pattern of reactions in leprosy. A clinical appraisal. Lepr. India **49** (1977) 221–228.
17. Sen Gupta, V., Sinha, S. and Ramu, G. Immunological assessment of sera of leprosy patients. Lepr. India **51** (1979) 43–48.
18. Welsh, G. P., Fajardo, T. T., Jr. and Guinto, R. S. Immunoglobulins and complement levels in lepromatous leprosy in special relation to ENL and resistance. Int. J. Lepr. **41** (1973) 552. (Abstract.)
19. Wemambu, S. N. C., Turk, J. L., Waters, M. F. R. and Rees, R. J. W. ENL—a clinical manifestation of the Arthus phenomenon. Lancet **2** (1969) 933–935.
20. World Health Organization. Immunofluorescent tests for the detection of autoantibodies. Immunological Techniques, May 1969, pp. 1–12.