Polyethylene Glycol Precipitates in Serum During and After Erythema Nodosum Leprosum—Study of Their Composition and Anticomplementary Activity[1]

Kunal Saha, Asit K. Chakrabarty, Vinay K. Sharma, and Virender N. Sehgal[2]

Erythema nodosum leprosum (ENL) occurs at times in borderline lepromatous and lepromatous leprosy. Its clinical manifestations are well documented. Its pathogenesis, however, still remains speculative, and has been the subject of recent investigations. A study of circulating immune complexes (CIC) in this connection is interesting. Recently, 4% polyethylene glycol (PEG) has been widely used to precipitate CIC ([13]), but study of their composition has received little attention. Chakrabarty, *et al.* ([1]) purified CIC from lepromatous sera by using PEG precipitates and analyzed the purified CIC. They could identify IgG, IgA, C1q, C1s, and C-reactive protein in the CIC with monospecific antisera and radiolabelled protein A followed by autoradiography. We undertook the present study to highlight CIC composition during and after ENL using the PEG precipitation technique.

MATERIALS AND METHODS

In all, 20 ENL patients, 14 with lepromatous (LL) and 6 borderline lepromatous (BL) leprosy, with duration varying from 1–11 years were included in the study. The diagnosis in these cases was made according to the well-defined criteria of ENL, keeping in view the outline of the Ridley and Jopling classification ([7]).

To control the ENL, 11 out of 20 patients were given prednisolone in a tapering dose of 30 mg to 5 mg daily; 3 received clofazimine (300 mg daily); 4 received chloroquine tablets (450 mg, 300 mg, and 150 mg daily for the first, second, and third week, respectively); 1 was given aspirin tablets (650 mg three times daily), and the remaining 1 case was given both aspirin and chloroquine. The duration of treatment varied from 1–2 weeks, and serum samples were taken before and four weeks after starting treatment.

Blood was collected aseptically from each patient by venipuncture. The first portion of the blood sample was discarded to prevent any contamination by skin bacteria over the cubital fossa, and the first syringe was replaced by a second one. Sera were separated under sterile precautions and stored at −20°C. Some fresh sera were also tested. Two-tenths ml serum, either stored or fresh, was placed in a sterile test tube; 0.2 ml 8% polyethylene glycol (MW 6000, British Drug House, England) was added to it and thoroughly mixed. The precipitate, thus formed, was separated by centrifugation in a clinical centrifuge and washed three times with 4% PEG.

The composition of the precipitate was determined qualitatively by the double-diffusion technique on agar gel using various monospecific antihuman immunoglobulin (heavy chain specific) and antihuman C3 antisera (Meloy Laboratories, Springfield, Virginia, U.S.A.). Antinuclear (ANF) and rheumatoid factor (RF) were also recovered in some PEG precipitate samples. For the demonstration of the antinuclear factor, an immunofluorescent technique was employed using buffy coat as the substrate ([12]). Rheumatoid factor was detected by the standard technique using RF latex test kits

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[2] K. Saha, M.Sc., M.B.B.S., Ph.D., F.A.A.A., Professor of Allergy and Immunology, Vallabhai Patel Chest Institute, Delhi University, Delhi, India. A. K. Chakrabarty, M.Sc., Ph.D., Reader, Department of Biochemistry, University College of Medical Sciences, New Delhi, India. V. K. Sharma, M.B.B.S., M.D., Assistant Professor, Department of Microbiology, Maulana Azad Medical College, New Delhi, India. V. N. Sehgal, M.B.B.S., M.D., M.A.M.S., Professor and Head, Department of Dermatology and Venereology, Maulana Azad Medical College and associated LNJPN and GB Pant Hospitals, New Delhi, India.

TABLE 1. *Qualitative analysis of composition of PEG precipitates during and after subsidence of ENL.*

Group	No.	Immunologic profile of PEG precipitates No. of samples							
		Immunoglobulins		Complement components			Autoantibodies and acute phase reactant		
		Any two	All three	Only C3	C3 and C4	C1q, C3 and C4	RF[a]	ANF[b]	CRP[c]
Normal subjects	15	3	2	5	1	0	0	0	0
Leprosy patients before starting treatment	20	9	11	1	6	12	2	2	5
Leprosy patients after completion of treatment (clinical cure of ENL)	20	1	19	1	7	10	1	1	4

[a] RF = rheumatoid factor.
[b] ANF = antinuclear factor.
[c] CRP = C-reactive protein.

(Laboratory Diagnostics Co., Morganville, New Jersey, U.S.A.).

PEG precipitates were studied quantitatively in 12 of the patients and in eight of the controls. The total protein contents of the PEG precipitates were determined by the biuret reaction ([2]). IgG, IgA, IgM, C1q, C3, and C4 levels in the PEG precipitates and in the respective whole sera were determined by single radial immunodiffusion techniques using monospecific antisera and their reference standards (Meloy). For the estimation of C1q levels, the WHO reference standard serum 67/97 was used as the C1q standard. All data were grouped and analyzed statistically. The amount of each immunoprotein in the PEG precipitates was also expressed as the percentage of its total amount present in the whole serum samples.

Anticomplementary activity of the PEG precipitates was assayed in seven lepromatous patients and in three normal subjects using the technique described by Opferkuch, *et al.* ([6]).

RESULTS

The qualitative and quantitative composition of PEG precipitates in sera of normal subjects and during and after ENL is shown in Tables 1 and 2, respectively. There was a statistically significant difference between the two groups.

The anticomplementary property of the PEG precipitates is shown in Table 3. The complement consumption capacity was found to be variable. However, it was remarkably more in the samples obtained from the lepromatous sera than in the normal sera, and it was independent of the presence of ENL. It neither depended on the amount of PEG precipitates in the sera, nor had any significant correlations with the composition of PEG precipitates. Also, C3 in the PEG precipitates had no influence on the anticomplementary action.

DISCUSSION

It has been shown that PEG usually precipitates all soluble CIC from serum, particularly when there is extreme antigen excess ([7]). But CIC in slight antigen excess are not completely precipitable since they can be detected in the supernatant after PEG precipitation.

The compositional profile of the PEG precipitates and autoantibodies of the sera of ENL patients (Table 1) is similar to that reported for various rheumatologic disorders ([5]). The prevalences of IgA (28%), IgM (28%), and C3 (45%) in PEG precipitates from the sera of systemic lupus erythematosis patients are high. The presence of C-reactive proteins in the CIC of lepromatous sera is interesting and confirms the earlier finding of Chakrabarty, *et al.* ([1]). It

TABLE 2. *Quantitative analysis of composition of PEG precipitates during and after ENL.*

Group	No.	Total protein (mg/ml) in PEG precipitates Mean ± S.D. (range)	Immunoprotein levels in PEG precipitates in Mean ± S.D. μg/ml serum (range) and as % of that in whole serum					
			IgG	IgA	IgM	C3	C4	C1q[a]
Normal subjects	15	0.092 ± 0.046 (0.025–0.161)	3 ± 10 (0[b]–40) 0.27%[c]	17 ± 30 (0[b]–80) 1.4%	7 ± 30 (0[b]–160) 0.71%	12 ± 20 (0[b]–60) 0.69%	14 ± 20 (0[b]–60) 4.0%	Not detected
Leprosy patients before starting treatment	12	1.83 ± 1.23 (0.45–4.25)	185.5 ± 139 (40–480) 11%	9.8 ± 9.0 (0[d]–28) 7%	36.3 ± 20.9 (16–80) 23%	17.3 ± 9.8 (0[e]–32) 17%	9.4 ± 7.7 (0[f]–19) 28%	6.6 ± 3.8 (0[g]–15.3) 4.9%
Leprosy patients after completion of treatment (clinical cure of ENL)	12	2.12 ± 1.36[h] (0.85–5.1)	159 ± 130.8 (23–433) 8%	9.8 ± 10.4 (0[d]–28) 8.7%	43 ± 36 (16–136) 23%	20.2 ± 21.9 (0[e]–82) 17%	6.7 ± 7.4 (0[f]–20) 19%	5.4 ± 3.1 (0[g]–9.5) 3.54%

[a] The C1q level is expressed as units in comparison with a WHO reference standard serum 67/97, taking it as 100 units per 100 ml.
[b] IgG, IgA, IgM, C3 and C4 were detected in 14, 5, 3, 5, and 8 normal samples, respectively.
[c] $\frac{\text{Concentration of IgG in the PEG precipitate}}{\text{Concentration of IgG in the whole sera}} \times 100.$
[d] IgA undetected in four samples before treatment and five samples after treatment.
[e] C3 undetected in one sample before treatment and one sample after treatment.
[f] C4 undetected in two samples before treatment and two samples after treatment.
[g] C1q was detected in four samples before treatment and four samples after treatment.
[h] $p = 0.055$, Student's t test, compared with leprosy patients before starting treatment.

TABLE 3. *Anticomplementary activity of PEG precipitates separated from control and lepromatous sera.*[a]

Name	Type of leprosy (during ENL and after remission)	PEG precipitate mg/ml serum	Microgram PEG precipitate added for 50% consumption of complement
Patients			
E.	LL (ENL)	0.95	90
B.D.	LL (ENL)	0.48	30
I.	LL	0.64	50
A.R.	LL (ENL)	0.95	16
S.L.	LL (ENL)	0.64	60
U.K.	LL	1.12	24
R.L.	LL (ENL)	1.29	56
Mean ± S.D.		0.87 ± 0.27	46.6 ± 23.6
Controls			
Ka		0.065	130
D		0.111	270
Ku		0.130	165
Mean ± S.D.		0.102 ± 0.027	171.7 ± 37.0

[a] Anticomplementary activity in the PEG precipitates from the lepromatous sera was remarkably more than that of the similar samples obtained from normal sera. Units of CH_{50} taken for the complement consumption test were 8.5 units.

is known that C-reactive protein (CRP) can activate complement not only via the classical pathway but also through the alternative pathway. It can also inhibit T lymphocytes and can modulate acute inflammatory processes by interfering with prostaglandin metabolism ([3]).

It is apparent from the present study that circulating immune complexes do not clear from the circulation despite marked amelioration in the clinical expression of ENL. Our earlier study had, however, demonstrated a significant rise of serum IgG ([11]) and C3 ([9]) levels following clinical remission of ENL. In another study, factor B split products (Ba) ([10]) were observed in ENL. Our findings of CIC levels are in keeping with those of Kemler and Alpert ([4]) who made a similar observation in cases of inflammatory bowel diseases on steroid treatment. Our data, therefore, raise the possibility of the failure of clearance of the CIC from the circulation even after the clinical subsidence of ENL, although a decrease in complement activation can still be found. These complexes may inhibit cell-mediated immune responses directed at *Mycobacterium leprae*-infected cells in a manner similar to the blocking phenomenon occuring in malignancy, which may result in profound anergy, thus worsening the condition. Furthermore, it is likely that CIC may impair the macrophage function of bacterial clearance by getting attached to T cells by Fc receptors. Our study also clearly indicates that the role of immunosuppressive drugs in ENL is only ephemeral and is not necessarily curative.

SUMMARY

Polyethylene glycol (PEG) precipitates obtained from sera were characterized and quantitated in 20 borderline lepromatous (BL) and polar lepromatous (LL) patients with moderate to severe erythema nodosum leprosum (ENL) before and four weeks after treatment with anti-ENL drugs, e.g., cortisone, clofazimine, chloroquine, or aspirin. Although the clinical severity of ENL subsided, there was no significant clearance of material that precipitates with PEG from the circulation. These precipitates had variable anticomplementary properties.

RESUMEN

Se cuantificó y caracterizó el precipitado obtenido con polietilén glicol (PEG) a partir de sueros de 20 pacientes con lepra lepromatosa intermedia (BL) y lepra lepromatosa polar (LL) con eritema nodoso leproso (ENL) moderado a severo, antes, y cuatro semanas

después del tratamiento con drogas anti-ENL tales como cortisona, clofazimina, cloroquina o aspirina. Aunque la severidad clínica del ENL disminuyó considerablemente o desapareció, no hubo una depuración significante del material circulante precipitable con PEG. Estos precipitados tuvieron propiedades anticomplementarias variables.

RÉSUMÉ

On a étudié les caractéristiques, et on quantifié les précipités de polyethylène glycol (PEG) obtenus à partir d'échantillons de sérum chez 20 malades atteints de lèpre lépromateuse borderline (BL) et de lèpre lépromateuse polaire (LL) souffrant d'un érythème noueux lépreux (ENL) modéré à assez sévère, avant d'un traitement avec des médicaments utilisés pour traiter l'ENL, de même que quatre semaines après ce traitement. Ces médicaments comprenaient la cortisone, la clofazimine, la chloroquine, et l'aspirine. Bien que la gravité clinique de l'ENL fut diminuée, il n'y avait pas de nettoyage significatif du matériel précipitant le PEG de la circulation. Ces précipités présentaient des propriétés anticomplément variables.

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