Preliminary Investigations on Abnormal Immunoglobulin(s) in Leprosy[1]

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In leprosy intimate, prolonged interactions between the virulent molecules of *Mycobacterium leprae* and the sensitive host cells, and a massive invasion of tissues by the bacteria may be expected to affect adversely the cells potentially capable of synthesizing immunoglobulins. Although immunodiffusion studies on the level of the different immunoglobulins in leprosy ([6]) have only revealed a twofold increase in each class of immunoglobulin (except IgD) over the normal level, no physico-chemical abnormality in the immunoglobulins was detected by the methods used.

As a consequence of our studies ([2,3]) on the host-parasite interactions in leprosy and our hypothesis related to the evolutionary molecular mechanisms of the interactions, we have now investigated the possibility that this intimate, prolonged contact might have caused cell transformations leading to the synthesis of abnormal immunoglobulin(s).

MATERIALS AND METHODS

Source of sera. Human sera employed consisted of 100 human sera obtained from different forms of advanced leprosy, 30 human sera from apparently healthy subjects, 20 sera from different forms of leukemia, 20 sera from syphilis, and 20 sera from tuberculosis. Both original and formalinized sera maintained at 20°C were used. Formalin was added to a final 0.1% concentration.

Commercial rabbit antisera, purchased from Behring Diagnostics, Montreal, were as follows: Anti-IgA, IgD, IgE, IgG and IgM antisera, antisera against κ and λ light chains, and an antiserum against Fab fragment of the IgG molecule. The identity of the commercial antisera was pre-examined against corresponding, purified immunoglobulins using the immunoelectrophoresis test ([4]). Two different buffers employed for both the agarose gel and electrophoresis chambers were: 1) an 0.075 M, pH 8.8 TRIS-barbiturate buffer, and 2) an 0.005 M, pH 8.2 barbiturate buffer containing 0.85% NaCl.

Immunoelectrophoresis. For immunoelectrophoresis, glass slides (1 × 6 inches) were first coated with a very thin underlay of 1.5% melted Indubiose and then overcoated with 1% melted Indubiose, up to the rim of plastic slide holders (Gelman Instrument Co., Ann Arbor). The patterns for wells and troughs were made by means of a standard punch.

Before electrophoresis, the wells were filled with 5 μl of a serum (a leprous serum being always placed opposite to a control serum), and then the plastic trays holding the slides were placed in a Deluxe Electrophoresis Chamber (Gelman Instruments Co.), cooled with running tap water. A constant current of 3.5 mA per inch of the agarose coated slide was applied for two hours, with the voltage rising to 450 volts. When the 8.2 barbiturate buffer was used, a current of 5 mA was applied for four to five hours.

After the desired time, the plastic trays were removed from the tank, and the central troughs emptied of agarose gel were filled with 0.1 ml of an appropriate rabbit antiserum. The frames were then placed in wet plastic tanks and left for 48 hours at 37°C. Then, after a preliminary inspection of the gels, the agarose slides were soaked overnight in 1 M NaCl at room temperature and dried at 37°C. The dry films were subsequently stained for three minutes in 0.1% Ponceau S dye solution made in 3% aqueous trichloracetic acid, destained in 7% acetic acid and inspected at 5X magnification. The counterimmunoelectrophoresis was carried out according to Remington's method ([5]). Each test was repeated two to three times.

The Rubino test. The assay was carried out according to the technic modified by Bier

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and Arnold ([1]).

Preparation of the unusual immunoglobulin. Twenty Rubino-negative leprous sera which, when examined by immunoelectrophoresis revealed the presence of an immunoglobulin termed IgK, which differed from the known Ig classes, were pooled. The immunoglobulin was then isolated from the pooled sera by the combined methods of ethanol fractionation, chromatography on A-50 coarse DEAG Sephadex, precipitation with 0.5 M zinc acetate and filtration through Sephadex G-200 gel as described by Skvaril and Brumelova ([7]). For the last portion of the procedure, 2 ml of the sample of the partly purified immunoglobulin was placed on a 100 ml-volume column, equilibrated with phosphate buffered saline (0.15 M NaCl, 0.01 M potassium phosphate, pH 7.4).

Absorption. In order to remove all known immunoglobulins from the above Ig preparation, aliquots of the very early eluate containing IgK and concentrated ten times by vacuum dialysis, were mixed with an equal volume of pooled anti-IgA, IgG, IgE, IgD and IgM twice concentrated antisera. The mixtures were agitated at 37°C for one hour and then centrifuged twice at 18,000 g for 20 minutes. The supernatant was examined for possible reactions with the above antisera by means of counterimmunoelectrophoresis and was considered satisfactory if no reaction was detected.

Production of antisera against the unusual immunoglobulin. The supernatant obtained and examined in the two preceding sections was injected intracutaneously into adult white rabbits: 0.5 ml aliquots were administered into four skin sites, and 0.2 ml were injected into four other skin sites within three weeks. After an additional three weeks the rabbits were bled. The immunoglobulins were precipitated from their sera with ammonium sulfate at 33% concentration, pH 7.8. The sediment was dissolved in distilled water, vacuum dialyzed at 4°C and reconstituted to one-half original serum volume with the phosphate buffered saline.

RESULTS

Only 32% of the sera from leprosy but none of the other human sera were found to react with formolized rabbit or sheep blood cells in the Rubino test. All the human sera formed regular precipitin patterns when assayed by the immunoelectrophoresis procedures with antisera against IgG and IgM, but none reacted with anti-IgD sera (Table 1). Thirty-five percent of Rubino-positive and negative leprous sera, but no control serum, reacted with anti-IgE antiserum.

When examined by the immunoelectrophoresis method, with anti-IgA antisera, the Rubino-negative leprous sera produced a long precipitation band that extended from the α_2 to the β_2 region (Fig. 1), contrasting with the usual IgA positions of all the other sera, including Rubino-positive leprous sera. The unusual behavior of this immunoglobulin, found only in Rubino-negative sera, was especially noticeable when 0.075 M, pH 8.8 TRIS-barbiturate buffer was used for immunoelectrophoresis. Under these conditions the abnormal immunoglobulin was posi-

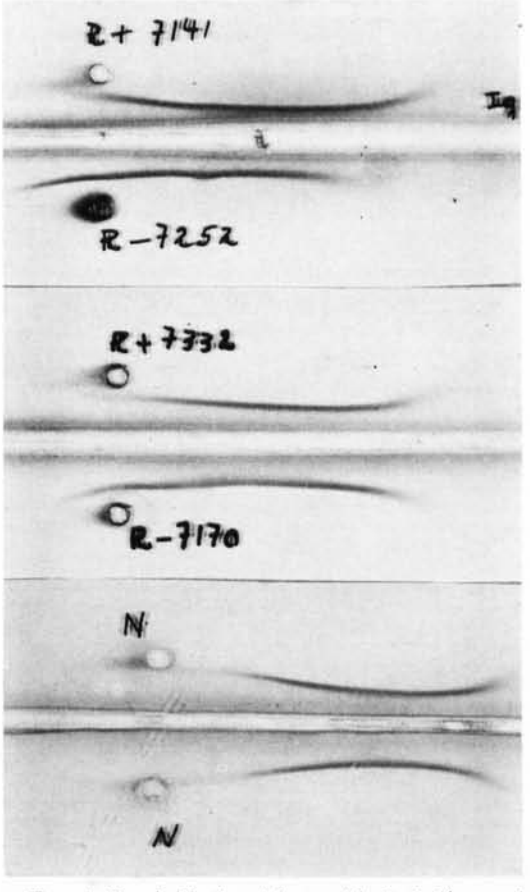

FIG. 1. Precipitin band formed in Indubiose gel by human sera assayed against IgA-antiserum on immunoelectrophoresis in a pH 8.2 barbiturate buffer. R+ and R- indicate Rubino-positive and Rubino-negative leprous sera, respectively; N indicates normal serum.

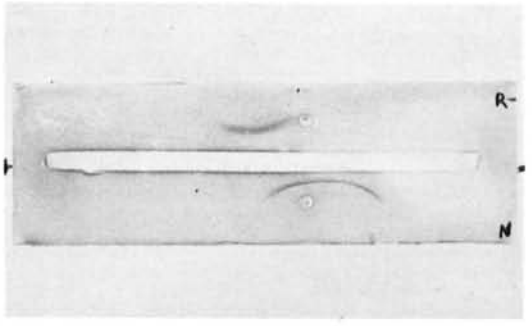

FIG. 2. Precipitin band produced on immunoelectrophoresis, in pH 8.8 TRIS barbiturate buffer, by human sera assayed with antisera against kappa chains. R-, Rubino-negative, leprous serum; N, normal serum.

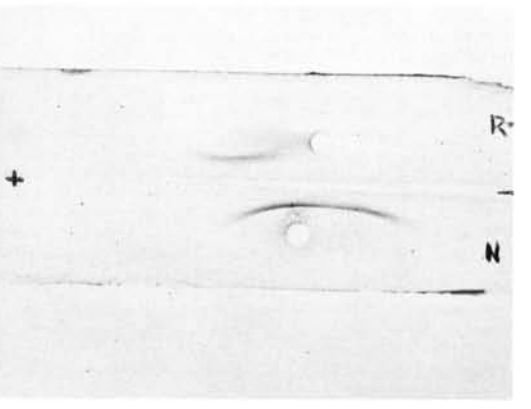

FIG. 3. Precipitin band formed on immunoelectrophoresis, in pH 8.8 TRIS-barbiturate buffer, by human sera examined with anti-IgG-Fab antiserum. R-, Rubino-negative serum; N, normal serum.

tioned in the area adjacent to the well, on the anode side (Fig. 2), in contrast with the normal IgA of the other human sera which were found on the cathode side of the well. In the same assay conditions, Rubino-negative leprous sera formed precipitin bands at 0.5 cm, 1.0 cm and 1.0 cm distances on the anode side when reacted with antisera against κ-polypeptide chains and Fab fragment of IgG (Table 1). In contrast, bands produced by other human sera were situated on the cathode side. Whereas the control sera reacted normally with an antiserum against λ-polypeptide chains, the Rubino-negative sera did not react with this antiserum. No easily discernible differences were found on straight electrophoresis of the human sera. Examined with the absorbed anti-IgK rabbit antisera, only the Rubino-negative leprous sera formed a faint precipitation band in an area adjacent to the well on the anode side.

DISCUSSION

The above reported data suggest that the IgK is an abnormal, negatively charged immunoglobulin whose Fc fragment appears to be partly antigenically related to the Fc and Fab fragments of IgA and IgG molecules. However, its behavior in the electric field at pH 8.8 differs from that of IgA and κ-chain molecules. The IgK molecule seems to possess only a single type of light chain that is

TABLE 1. *Reactions (+) of human sera with rabbit anti-immunoglobulin antisera, revealed by immunoelectrophoresis.*

Human serum from	Absorbed anti-IgK antiserum	Anti-antiserum	Anti-κ		Anti-IgA		Anti-IgG-Fab		Anti-IgG, IgM	Anti-IgD
			Anode	Cathode	Anode	Cathode	Anode	Cathode		
Leprosy (Rubino −)	+	−	+		+		+		+	−
Leprosy (Rubino +)	−	+		+		+		+	+	−
Leukemia	−	+		+		+		+	+	−
Tuberculosis	−	+		+		+		+	+	−
Syphilis	−	+		+		+		+	+	−
Gonorrhea	−	+		+		+		+	+	−
Healthy subjects	−	+		+		+		+	+	−

partly antigenically related to the κ-polypeptide chains but not to λ-chains. At least one site in the IgK molecule is antigenically unique as shown by its examination with absorbed anti-IgK antiserum.

We tentatively postulate that the IgK molecule is entirely different from all known classes of immunoglobulins. Its structure may consist of two heavy κ-polypeptide chains and two κ_l light polypeptide chains.

It cannot be excluded, however, that the κ_l light chains have not been assembled with heavy chains, and that only few or no Fab subunits are being synthesized for an IgK molecule. The latter event might be correlated with the apparent lack of an inhibitory effect of leprous immunoglobulins on *Mycobacterium leprae*.

The occurrence of an abnormal, and possibly defective immunoglobulin, may depend on a transformation of certain, potentially immunoglobulin-synthesizing cells by the agent of leprosy. Alternatively, an intimate contact between the etiologic agent of leprosy and nascent (or mature) IgA molecules might cause their partial depolymerization and fragmentation, leading to the occurrence of the abnormal Ig molecules.

SUMMARY

A new, abnormal immunoglobulin designated IgK has been discovered in leprous Rubino-negative sera. The IgK having a negative net charge and its own antigenic specificity appears to be partly related to the Fc fragment of IgA and to Fab fragments of known immunoglobulins, but its net charge is negative at pH 8.8. Its molecule seems to possess kappa but not lambda light polypeptide chains. Implications of this discovery are discussed.

RESUMEN

Se comunica el descubrimiento de una nueva inmunoglobulina anormal, denominada IgK, en el suero de pacientes con lepra Rubino-negativos. La IgK, que tiene una carga neta negativa y su propia especificidad antigénica, parece estar parcialmente relacionada con el fragmento Fc de la IgA y con los fragmentos Fab de las inmunoglobulinas ya conocidas pero su carga neta es negativa a pH 8.8. Su molécula parece poseer cadenas ligeras kappa pero no lambda. Se discute la importancia de este descubrimiento.

RÉSUMÉ

On a découvert une nouvelle immunoglobuline normale dans des échantillons de sérum de malades de la lèpre, négatifs à la réaction de Rubino. Cette immunoglobuline a été désignée par le sigle IgK. L'immunoglobuline IgK a une charge nette négative. Sa propre spécificité antigénique semble être liée en partie au fragment Fc de l'IgA et aux fragments Fab des immunoglobulines connues. Mais sa charge nette est négative au pH 8.8. Sa molécule paraît contenir des chaînes polypeptidiques légères kappa, mais pas de chaînes lambda. Les conséquences de cette découverte sont discutées.

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REFERENCES

1. Bier, O. and Arnold, K. Über die Serologie der Lepra; die Spezifität der Rubino-Reaktion. Arch. Schiffs. Tropen-Hyg. **39** (1935) 231-236.
2. Kwapinski, G. and Kwapinski, E. Immunological reactions of *Mycobacterium leprae* and *Mycobacterium lepraemurium* grown in cayman. Can. J. Microbiol. **19** (1973) 764-766.
3. Kwapinski, G., Kwapinski, E. and McClung, N. M. The growth of *M. leprae* in snakes. Can. J. Microbiol. **20** (1974) 420-422.
4. Laurell, C. B. Electrophoretic microheterogeneity of serum α_1-antitrypsin. Scand. J. Clin. Lab. Invest. **17** (1965) 271-274.
5. Remington, J. S., Gaines, J. D. and Gilmer, J. A. Demonstration of Candida precipitins in human sera by counterimmunoelectrophoresis. Lancet **I** (1972) 413-419.
6. Sirishina, S., Charupatana, C. and Ramasoota, T. Serum immunoglobulins in leprosy patients with different spectra of clinical manifestations. Proc. Soc. Exp. Biol. Med. **140** (1972) 1062-1068.
7. Skvaril, F. and Brumelova, V. Immunochemical comparison of peptic fragments of Fab and Fc fragments of normal human gamma globulin. J. Hyg. Epidemiol. Microbiol. Immunol. **9** (1965) 265-269.