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Anti-γ-Globulin Activity in Leprous Lesions

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INTRODUCTION

Leprosy is one of the chronic infectious diseases which may quite often show positive reactions for rheumatoid factors in serum ([2, 3, 6]). The incidence of positive reactions varies according to the methods used and category of the disease. In lepromatous leprosy Bonomo and Dammacco ([2]) (1968) found the latex slide test positive in 48 per cent and the sensitized sheep cell test positive in 30 per cent. In tuberculoid leprosy the corresponding percentages were 7 and 3.3.

Some other autoimmune antibodies, such as antinuclear factors and thyreoglobulin antibodies, are also often found in patients with leprosy. These results, and some of the clinical manifestations, have led some authors to consider leprosy a unique model for the study of autoimmune states such as diseases of connective tissue and related disorders.

Our interest in anti-γ-globulin in leprosy came as a result of observations made by Milde and Tönder in 1968 ([7]). Employing the mixed agglutination technique they found anti-γ-globulin activity in the granulomatous inflammation in the joint capsules in rheumatoid arthritis. The activity was present even in cases with normal amounts of rheumatoid factors in serum. This focused interest on the diseased tissue, and other granulomatous inflammations were studied, giant cell arteritis ([12]), tuberculosis and leprosy ([13]), and similar results were achieved. Our preliminary studies of leprous lesions have been extended and the results are presented here.

MATERIAL AND METHODS

Tissue. Fifty-one biopsies representing various lesions from 24 patients were examined: lepromatous leprosy, 14; erythema nodosum leprosum, 6; dimorphous leprosy, 3; indeterminate leprosy, 1. Biopsies were frozen immediately after removal and sent in freezing bags with dry ice from Jerusalem to Bergen. Cryostat sections of the tissue were prepared and mounted on cover-glasses as described previously([10]). A large series of sections were cut for mixed agglutination and in each series some sections were selected, mounted on slides for histologic examination and control of the stage of the lesions. Hematoxylin and eosin and Ziehl-Neelsen stains were used. In cases where enough material was available the tissue was embedded in paraffin for further histologic study.

Sera. Sera were obtained from the patients at the same time as the biopsies were taken. Antisera to sheep erythrocytes were produced by immunization of rabbits. Rabbit antisera specific to human γG-, γM-, and γA-globulins respectively, as well as a polyvalent antiserum to γ-globulin and complement, were prepared as described previously ([4, 11]). Rat antiserum to C1q was kindly supplied by Dr. Thunold, Bergen.

γ-globulins. Human γ-globulin (Fraction II 16% solution) was purchased from AB Kabi, Stockholm. Rabbit γ-globulin (lyophilized Fraction II) was purchased from Hyland Laboratories, Los Angeles, California. Denatured γ-globulin was prepared by heating 4 per cent solutions at 63°C for 10 minutes.

Erythrocytes. Sheep erythrocytes were obtained from whole blood collected in Alsever's solution. Before use, the erythrocytes were washed four times in ten volumes of phosphate buffered saline, pH 7.2. They were finally packed at 1,000 x g for ten minutes.

Tests for rheumatoid factor in serum. The Waaler test was carried out as described elsewhere ([8]), except that the

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sheep erythrocytes were sensitized by the rabbit antiserum at a dilution four times higher than the agglutination titer instead of twice as high. The Latex (RA) test was performed as recommended by the manufacturer with reagents purchased from the Hyland Laboratories.

Indicator systems for mixed agglutination. Sheep erythrocytes were sensitized by various agglutinating and subagglutinating amounts of corresponding rabbit antiserum. Sensitization was performed by mixing equal volumes of a 1 per cent suspension of sheep erythrocytes and diluted antiserum. The mixture was left at room temperature for ten minutes. After washing, the erythrocytes were made up to a 1 per cent suspension in the phosphate buffer containing 2 per cent NaCl ([7]).

Mixed agglutination with tissue sections. The sections were used either without pretreatment or were treated with various reagents as will be described below. The concavity of microculture slides was filled with indicator cells and sealed by the cover-glass with the tissue section on it. The slides were inverted, and left for 30 minutes at room temperature. The slides were then turned cover-glass up and left for 20 minutes. Usually slight tapping of the glass around the tissue section was necessary to accelerate the detachment of the erythrocytes from the glass before reading.

Reading. This could be done macroscopically, but preparations were always examined microscopically at 20-fold and 80-fold magnification to verify whether or not the erythrocytes were attached to the tissue. In positive reactions the sensitized red cells will stick to the tissue sections and in strong reactions cover the reactive areas almost completely. In the weaker reactions more scattered foci of adhering cells were observed. In negative reactions the erythrocytes do not stick to the tissue, but fall to the bottom of the incubation chamber.

Grading of the reaction. Using different sensitizing doses it is possible to titrate the anti-γ-globulin activity in the tissue. The erythrocytes were sensitized with 4, 2, 1, ½, ¼ and ⅛ agglutinating units of antiserum. The tissue was considered strongly reactive (3+) when positive reactions were obtained with cells sensitized with subagglutinating amounts of antiserum, moderately reactive (2+) when the postive reactions stopped at 1 agglutinating unit, and weakly reactive (1+) when 4 and 2 agglutinating units were needed to give the reaction.

RESULTS

Mixed agglutination. Fifty-one biopsies were examined; 31 showed positive mixed agglutination. The results obtained with one of our biopsies are illustrated in Fig. 1, a case of reactivation of leproma. As can be seen from the pictures, the mixed agglutination is positive in the area of the leprous granuloma, with sensitized cells adhering to the tissue section. Nonsensitized cells do not adhere and antiserum to γM blocks the reaction. Another positive reaction from a case of lepromatous leprosy, which clinically was stated to be in the stage of resorption, is shown in Fig. 2. The hematoxylin and eosin section showed numerous small lepromas in the corium and the sensitized cells adhered to areas of the granulomas. Antiserum to γG did not block the mixed agglutination reaction (Fig. 2C). Neither did antiserum to γA and γM inhibit the reaction, but antiserum to Clq gave complete inhibition.

The adherence of erythrocytes to the tissue did not take place in active lepromas if the rabbit antiserum was added first and nonsensitized cells afterwards. The antibody has to be bound to the erythrocytes before the mixed agglutination reaction can be carried out.

Erythema nodosum leprosum gave mixed agglutination in a certain number of cases. The distribution of the immunologic activity followed the inflammatory reaction in a similar way as in lepromas.

Correlation between clinical symptoms and signs, histology and mixed agglutination. *The general findings* are summarized in Table 1. Thirty-one biopsies from 15 patients gave positive mixed agglutination; 20 biopsies from 9 patients gave no reaction. All the positive biopsies except one came from cases with lepromatous leprosy. The one exception was a leproma from a patient with dimorphous leprosy. Of 29 lepromas from 14 patients, 21 biopsies from

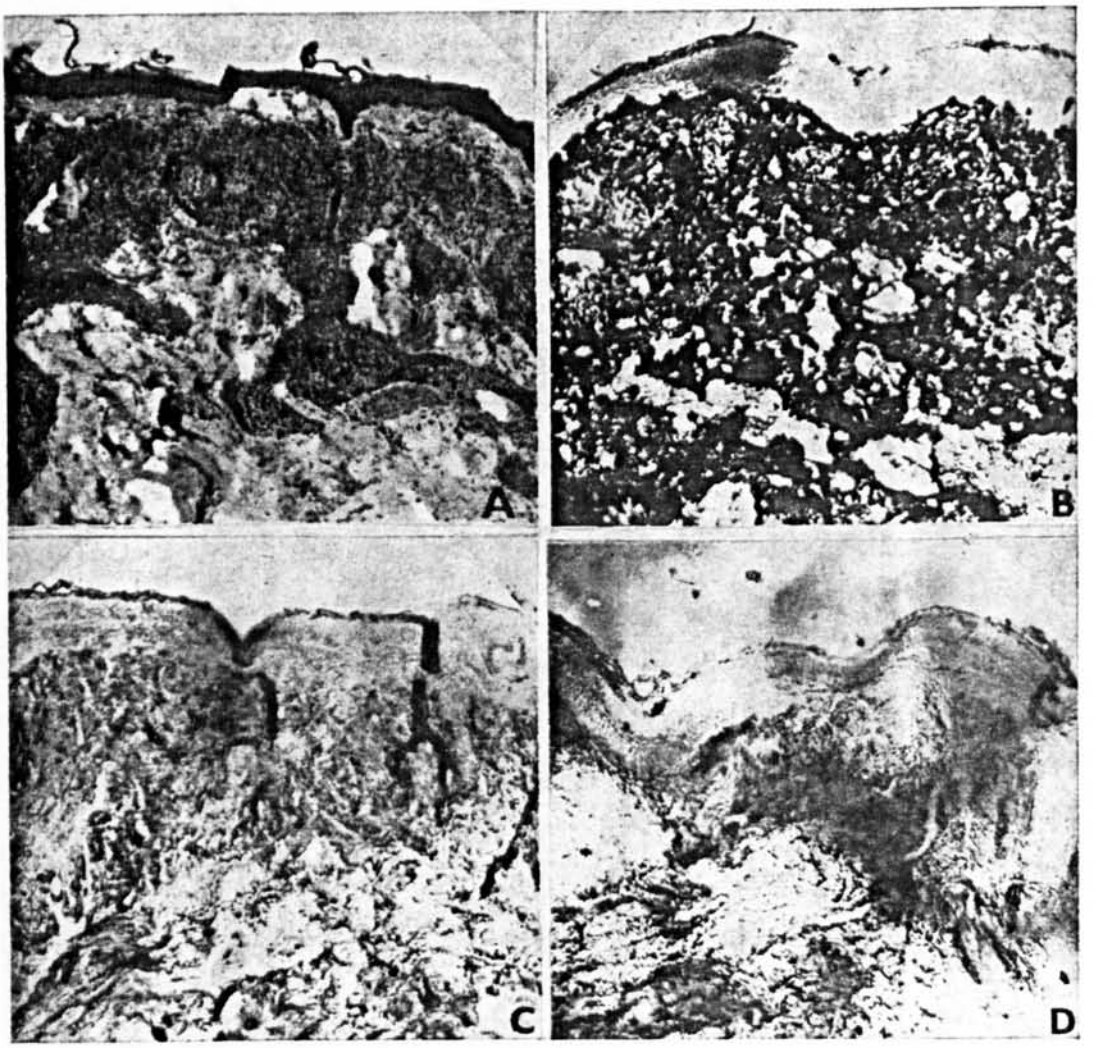

FIG. 1. Frozen sections of tissue from a case of lepromatous leprosy, reactivation of leproma. × 45. (A) Hematoxylin and eosin with diffuse to nodular granulomatous lesion in the dermis. (B) Mixed agglutination. The black area in the region of the dermis represents sensitized cells adhering to the tissue. (C) Nonsensitized cells do not stick to the tissue sections, and the contours of the unstained tissue can be seen. (D) Blocking test with anti-γ M serum. The frozen section is first incubated with antiserum, and thereafter sensitized cells are added, 2 agglutinating units. No adherence of the cells.

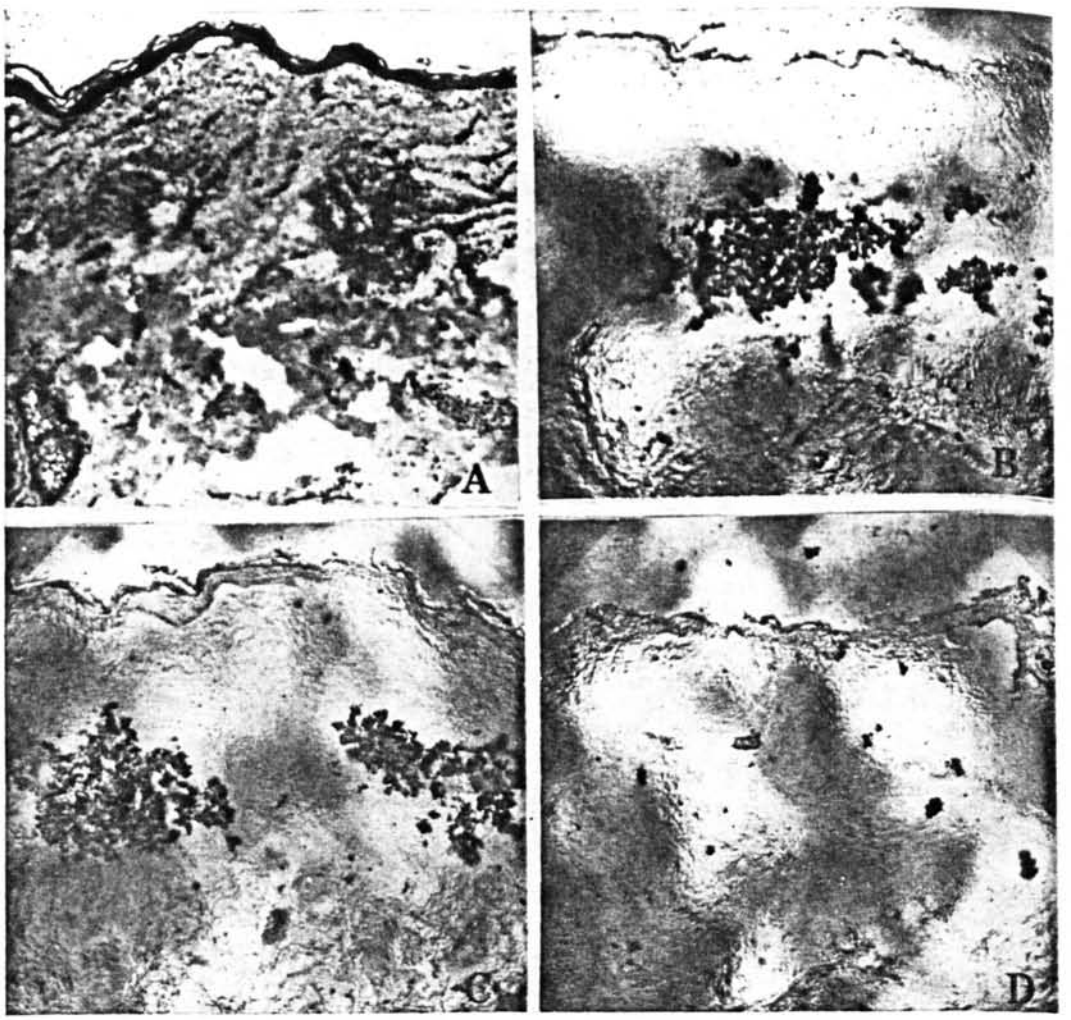

FIG. 2. Frozen sections of tissue from a case of lepromatous leprosy, leproma, clinically in regression. × 45. (A) Hematoxylin and eosin stain with small lepromas in a band in upper part of dermis. (B) Mixed agglutination. The sensitized cells stick to the areas where the lepromas are located. (C) Blocking test with anti-γ-G serum. The tissue section was first incubated with antiserum; thereafter sensitized cells were added. The sensitized cells adhere to the same areas as in Fig. 2B. No inhibition of mixed agglutination. (Antiserum to γM and to γA gave a similar negative result.) (D) Blocking test with antiserum to C1q. Similar procedure to that in 2C. The contours of the unstained tissue section are seen; no cells stick to the tissue section.

TABLE 1. *Mixed agglutination with tissue sections; 51 biopsies from 24 patients with leprosy.*

Mixed aggl.	Clinical diagnoses: Lepromatous leprosy: Lepromas	Clinical diagnoses: Lepromatous leprosy: Erythema nodosum	Indeterminate Dimorphous Tuberculoid	Total
Positive	21 (11)	9 (3)	1[a] (1)	31 (15)
Negative	8 (3)	5 (3)	7 (3)	20[b] (9)

Number of patients in brackets.
[a] Histology: Leproma-like lesion, a dimorphous case.
[b] Histology: Minor noncharacteristic inflammatory lesions, or small lepromas in regression.

11 patients gave positive mixed agglutination. Nine biopsies from patients with erythema nodosum gave positive mixed agglutination; 5 gave no reaction. As indicated in the table, the 20 biopsies which gave no mixed agglutination were from definitely regressing lesions with minor inflammatory activity.

Lepromas with strong mixed agglutination are derived from new active cases which have not been treated or treated for a short time. Histologically the lesions were fresh lepromas with nodular to diffuse cellular reaction with many lepra cells throughout the corium and many bacilli (Figs. 3A and 3B). Medium strong mixed agglutination was found in similar lesions, but in some there were signs of resorption both clinically and histologically. The weak mixed agglutination in lepromas was always found in cases stated to have definite clinical signs of resorption. The histology showed small perivascular lepromas or minor remnants of lepromas and some perivascular strands. Lesions which gave no mixed agglutination are from patients with definite clinical signs of resorption. Histologically there was nonspecific inflammation with perivascular strands and sometimes with remnants of lepromas.

On the whole we can state that in patients with the diagnosis leproma, the clinical activity of the disease, histologic picture and immunologic reaction showed striking correlation. There is, however, one patient in our series showing complete disagreement between the clinical data on the one hand and histologic and immunologic activity on the other. The clinical information

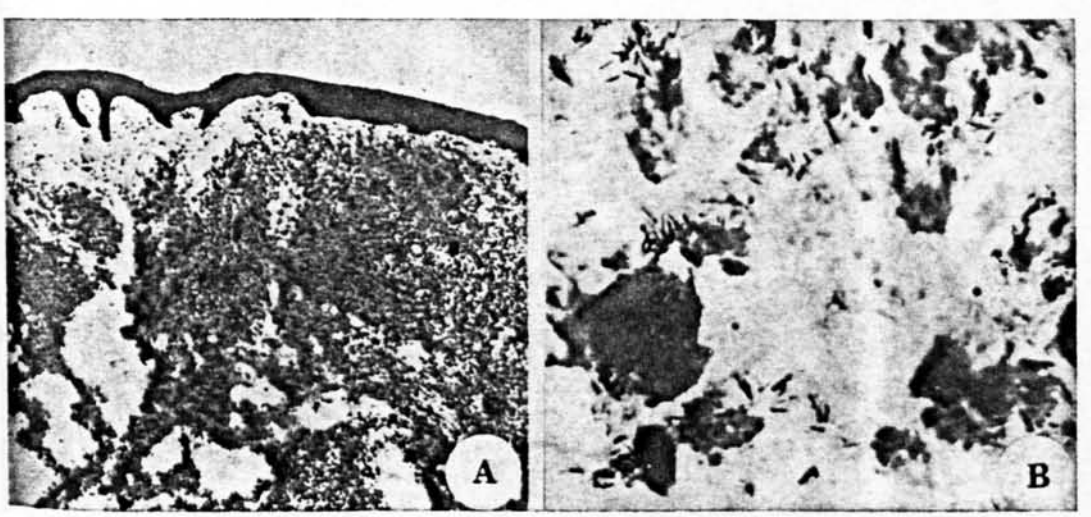

FIG. 3. Frozen section from a leproma to illustrate the histology in cases with a strong positive mixed agglutination. (A) Ziehl-Neelsen stain showing a diffuse lepromatous lesion throughout the corium. × 45. (B) Ziehl-Neelsen stain showing macrophages and numerous bacilli. × 1200.

TABLE 2. *Lepromatous leprosy, patient Z.V. Control after 4 months' treatment*

Biopsy date	Clinical	Anti-γ-globulin in tissue	Histology
November 1968	L.L. New patient	++	Leproma throughout whole dermis
May 1 1969 *a*	After 4 months' treatment	+γM	Small definite lepromata
b	2 lepromas in resorption	negative	Focal small lepromata

states that it is a new case of untreated leproma. Histologically there was a minor non-specific inflammation and mixed agglutination was negative. The specimen was apparently not representative, which is not unusual with small biopsies.

That treatment with clinical improvement is accompanied by regression of the histologic lesion and gradual disappearance of the immunologic activity is demonstrated in Table 2. After four months' treatment there were definite clinical signs of resorption followed by a similar change in histology. Mixed agglutination was weak in one biopsy, negative in another.

Erythema nodosum. The usual finding in the early lesions was strong or medium strong mixed agglutination, and focal lepromata with diffuse cellular infiltration throughout the dermis (Fig. 4). When the clinical condition changed and the lesions showed definite signs of improvement the mixed agglutination became negative and histologically only perivascular strands with few cells and in some cases insignificant remnants of lepromas were revealed.

There were however, exceptions to this rule. Clinical improvement may be reported without major changes in histology and with unaltered or minor changes in mixed agglutination. In one patient four biopsies were taken from the same lesion from April 27 to May 14. The first biopsy was clinically said to be from an early stage. The following two biopsies were stated to be in resorption, the third to be almost complete.

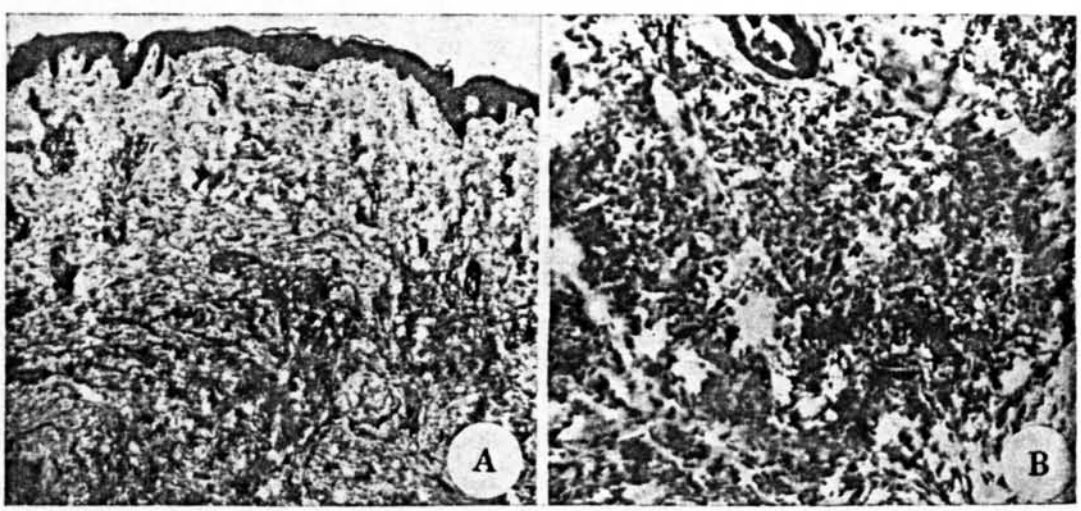

FIG. 4. Frozen section from a case of erythema nodosum leprosum to illustrate the histology in cases with a strong positive mixed agglutination. (A) Hematoxylin and eosin stain with remnants of lepromas, edema and scattered diffuse cellular infiltration. × 30. (B) Hematoxylin and eosin stain with marked diffuse cellular infiltration, mainly macrophages and some polymorphonuclear leucocytes. × 120.

TABLE 3. *Comparison of clinical state, histology and immunologic activity in biopsies from three patients with dimorphous leprosy*

Patient		Clinical	Histology	Immunology
B.E.		Leproma in resorption	Focal noncharacteristic inflammation	Negative
B.M.	*a*	Leproma	Small regressing lepromas	Negative
	b	Tuberculoid	Small perivascular and perineural infiltrates, noncharacteristic	Negative
K.J.	*a*	Tuberculoid lesions New patient not treated	Tuberculoid granulomas	Nonspecific reaction
	b	Leproma-like	Perivascular lepromas	+++ γM

ly resorbed. The histology and mixed agglutination revealed no remarkable change. The discrepancy may perhaps be caused by the biopsy trauma, inducing an artificial local activation not affecting the general condition of the patient.

Indeterminate leprosy is represented with one early case only. Histologically there was a minor nonspecific inflammation with perivascular infiltrates, and mixed agglutination was negative.

Dimorphous lesions. Five biopsies from three patients were examined (Table 3). Three of the biopsies showed minor inflammatory activity, small regressing lepromas in one and minor nonspecific inflammation in two instances, and all showed negative mixed agglutination.

Patient K. J. had one lepromatous lesion which gave a strong mixed agglutination reaction. The other lesion from this patient was clinically supposed to be tuberculoid. Histologically tuberculoid granulomas were found in the dermis, but in the mixed agglutination tests both sensitized and nonsensitized cells adhered to the tissue lesion. These results gave us no information about anti-γ-globulin activity in tuberculoid granulomas.

TABLE 4. *Comparison of tissue immunology and acid-fast bacilli in lesions.*

Anti γ-glob. activity	Bacilli	
	Positive	Negative
+++	14	0
++	9	1
+	4	3
−	8	8

Correlation, acid-fast bacilli and anti-γ-globulin in the tissue lesions. In Table 4 the anti-γ-globulin activity has been classified according to the strength of the reaction, and compared with the results of the examination for acid-fast bacilli. All 14 biopsies with strong positive mixed agglutination contained bacilli, and of the ten with medium-strong mixed agglutination only one was negative for bacilli. There was an increasing number of negatives for acid-fast bacilli in the two following groups. The results seem to indicate a positive correlation.

Inhibition tests with aggregated γ-globulin

Table 5 shows that in 20 biopsies inhibition of mixed agglutination was achieved with both human and rabbit γ-globulin. In three instances rabbit globulin only was active and in one case only human globulin gave inhibition of mixed agglutination. In two biopsies the results of the inhibition tests were not conclusive. The available material was too scanty to carry out complete tests with the necessary controls. However, our results indicate that the anti-γ-globulin activities in the lesions can be classified as rheumatoid factors.

TABLE 5. *Inhibition of anti-γ-globulin reactivity in leprous lesions. Effect of aggregated human and rabbit γ-globulin.*

Inhibition with aggregated γ-globulin	Number
Human and Rabbit	20
Rabbit alone	3
Human alone	1
Indefinite	2

Inhibition tests with antisera

Our first series was carried out with antiserum to γG and γM only. Table 6 shows that in 14 biopsies the activity was of γM class, in two cases of γG, but in two biopsies antiserum to both γM and γG had to be used in order to get inhibition of mixed agglutination.

In our second series a polyvalent antiserum to γ-globulin and complement as well as antisera to γA and C1q were included in the inhibition experiments. As can be seen from Table 7 the results are somewhat different from those of the first series. None of the mixed agglutination tests were inhibited by antiserum to γM. With five of the eight specimens anti-γ-G serum gave complete inhibition (biopsy Nos. 3, 4, 5, 6 and 7). In two cases (Nos. 4 and 6) this antiserum acted alone; in other instances there was partial inhibition with antiserum to γA (No. 7), and C1q serum (No. 5), and with antiserum to both γA and C1q in two instances (Nos. 1 and 3). In two biopsies antiserum to C1q alone inhibited the reaction (Nos. 2 and 8). Thus it appears that all three classes of γ-globulins, as well as complement, may be responsible for the anti-γ-globulin activity in the tissue sections.

Long term observations

Some patients have been controlled with biopsies for 1½ years, and some interesting

TABLE 6. *Class of anti-γ-globulin activity in leprous lesions (first series).*

γM	14
γG	2
γM + γG	2

TABLE 7. *Mixed agglutination—Effect of incubation of tissue sections with various antisera (second series).*

Biopsy no.	Saline control	Tissue sections after incubation with antiserum to: γ-glob. and C	γM	γG	γA	C1q	Class of activity
1	++	−	++	+	+	+	γG, (γA, C)
2	++	−	++	++	++	−	C
3	+++	−	+++	−	+	+	γG, (γA, C)
4	++	+	++	−	+	++	γG
5	+++	−	+++	−	++	+	γG, (C)
6	+++	+++	+++	−	+++	+++	γG
7	+++	−	+++	−	+	+++	γG, (γA)
8	+	−	+	+	+	−	C

changes were observed.

Patient C.D. in April 1969 showed an erythema nodosum lesion in beginning resorption which had a medium-strong mixed agglutination reaction. The immunologic activity was due to γ-globulin of both γG and γM class. In February 1970, when there was no lepra reaction, two fresh lepromata were removed. Both gave a medium-strong mixed agglutination reaction. In one the activity was of γG class; in the other complement also was present.

Patient M. C. in April 1969 had a lepromatous lesion with immunologic activity of γM class. Eleven months later another biopsy (histologically of the same type as the first) had complement as the factor responsible for the positive mixed agglutination.

Thus the class of anti-γ-globulin activity may vary from time to time, but also different biopsies taken on the same day in one individual may show variations. The inflammatory lesions therefore behave as if they were autonomous.

Comparison of anti-γ-globulin activity in tissue and serum

In 24 cases we have results from both serum and biopsy. Twelve gave positive mixed agglutination, and the other 12 biopsies were negative. As can be seen from Table 8, positive and negative reactions for rheumatoid factors in serum may be found both in the "tissue-positive" and the "tissue-negative" group, and approximately in the same number. These results also indicate the apparent autonomy of the immunologic activity of the tissue lesions.

TABLE 8. *Comparison of anti-γ-globulin activity in tissue and serum.*

	Serum	
Tissue	Pos.	Neg.
Positive: 12	3	9
Negative: 12	2	10

Immunologic activity in lepromin reactions

Altogether ten biopsies from seven individuals were examined (Table 9). Three biopsies gave negative mixed agglutination. (Patient 617, G.M.; 618, K. J.; and contact T. A., 655). The histology in these three cases revealed minor cellular infiltrates, and the material used for frozen sections was probably not representative for the lesions. Seven biopsies gave positive mixed agglutination. The strong and medium-strong mixed agglutination reactions were found in lesions with definite granulomatous inflammation and in cases with lepromin reactions noted to be strong. Weak positive anti-γ-globulin reactions were present in cases with more moderate inflammatory lesions and the lepromin reactions were also noted to be weaker in these two cases.

Another interesting detail should be pointed out with No. 618, K.J. This patient is the same as the K.J. mentioned in Table 3. The leproma in this patient with dimorphous leprosy examined in May 1969 gave a strong mixed agglutination with activity of γM class. In November 1969 the lepromin reaction was carried out. This stimulus produced neither a strong lepromin reaction, definite granulomatous inflammation nor immunologic activity in the lesion.

Rheumatoid factor in serum was positive in only one of the patients (No. 616, R. R.) The other six individuals were "seronegative", regardless of the immunologic state of the tissue lesions.

Our findings indicate that the reason for our previous negative results on anti-γ-globulin in tuberculoid lesions is to be found in the inflammatory state at the time of examination. If active inflammation is induced, these patients reveal immunologic activities similar to patients with lepromatous leprosy. Our results in lepromin reactions also indicate the immunologic autonomy of the individual lesions.

DISCUSSION

Employing the technique of mixed agglutination with tissue sections, we have demonstrated that sections of active lesions

in lepromatous leprosy (lepromas and erythema nodosum leprosum) react with sheep erythrocytes sensitized by rabbit antiserum. This activity disappears with regression of the lesions. The mild spontaneous inflammatory lesions we have examined in lepromatous, dimorphous, and indeterminate leprosy showed no such activity. However, some tissue sections from biopsy specimens of lepromin reactions from patients with tuberculoid leprosy, dimorphous leprosy, and one contact gave positive reactions.

No reaction was obtained with nonsensitized erythrocytes, except for a nonspecific reaction with a tuberculoid granuloma. Nor was any reaction achieved if the sensitizing serum was added alone first and nonsensitized erythrocytes afterward. This indicates that the reactions obtained in lepromatous leprosy were dependent on the rabbit antibody adherent to the erythrocytes. The activity in such tissues is accordingly similar to the activity detected in rheumatoid tissue([7]).

Further evidence for the dependence of tissue activity on anti-γ-globulins similar to rheumatoid factor was obtained from inhibition experiments. Isolated γ-globulin (Fraction II) of human and/or rabbit origin and particularly denatured γ-globulin, inhibited the reaction. Whole human or rabbit serum gave no inhibition. This type of reaction is one of the criteria for rheumatoid factor activity in an anti-γ-globulin reaction. However, Clq can also react with denatured γG-globulin or with γG-globulin appearing in an antigen/antibody complex, and accordingly C1q can act as a bridge in mixed agglutination with tissue sections. An antiserum to Clq was therefore included in the inhibition experiments.

TABLE 9. *Lepromin reaction. Clinical findings, histology, tissue immunology*

Patient		Diagnosis	Clinical findings	Lepromin reaction	Histology	Tissue immunology
616 R.R.	*a*	T.L.	Tuberculoid lesions in resorption	+++	Granulomata fibrinoid necr.	+++
	b			+++	Granulomata macrophages	++ γA
617 G.M.		T.L.	No active lesions	++	Very scanty* cellular infiltrates	Neg.
618 K.J.		D.L.	Tuberculoid and leproma-like lesions	+	Small infiltrates	Neg.
619 B.E.	*a*	T.L.	No active lesions	+++	Granulomatous lesions in both specimens	++ γA
	b			+++		++ γA
654 T.A.		Contact	No lesions	+++	Nodular granulomata	+++ Clq, γM
655 T.A.				+++	Scanty infiltrates, representative?	Neg.
657 A.C.		T.L.	No active lesions	++	Moderate perivascular infiltr.	+
658 N.S.		T.L.	No active lesions	+	Perivascular granulomas	+

* Frozen section not representative. Paraffin section: definite granulomata.

The results of the inhibition experiments, including specific antisera to γ-globulins and to C1q, showed that the activity detected by mixed agglutination was dependent on a very heterogeneous group of factors. Apparently the anti-γ- globulin activity was connected with γG, γM, γA, and C1q, either in combination or separate.

The lack of relation between the occurrence of anti-γ-globulins in the tissue lesions and increased amounts of rheumatoid factor in serum is not surprising. Similar observations have been made in studies of rheumatoid arthritis. The so-called "seronegative" patients may show strong activity in their affected synovial tissue (7).

Whether the anti-γ-globulin activity in tissue is connected with serum factors or cellular components can not be settled conclusively on the basis of present experiments. However, the activity was not reduced by washing the sections in saline, indicating that the components responsible for the activity are firmly bound in the tissue. Since there was a definite correlation between activity and the presence of acid-fast bacilli in the lesions, some antigen/antibody complexes may be responsible. In other words, γG antibodies appearing in complex with the bacilli may have absorbed anti-γ-globulin factors from the tissue fluid, or the anti-γ-globulins may be produced locally by immunocytes in the lesions. The induction of rheumatoid factor-like activities in leprous lesions may follow a pattern similar to that in experimental hyperimmunization (1) and in renal allograft recipients (5).

Our long term observation, as well as the results with lepromin reactions, indicate a certain immunologic autonomy of the various inflammatory lesions. It is interesting to note that a normal contact and patients with tuberculoid leprosy with mild inflammatory lesions which regularly gave negative mixed agglutination, developed anti-γ-globulin activity in the induced active granulomas of their lepromin reactions. Is the inflammatory reaction a primary event in relation to the occurrence of anti-γ-globulin activity in the lesion? Does the anti-γ-globulin activity in the lesions, as in rheumatoid arthritis, interfere with the disease process? Or is the occurrence of anti-γ-globulin activity something which follows passively in the wake of the inflammatory reaction as a shadow follows us when we walk in the sun? What are the early and late events in anti-γ-globulin activity in these chronic granulomas? When does this activity occur in the spontaneous disease? Does it occur in the more active tuberculoid lesions? When does it occur in the lepromin reaction and when does it disappear? It is probably premature to discuss these problems. Our findings have raised more questions than they have answered.

The production of anti-γ-globulin and their binding to granulomatous inflammatory tissue reveal data which have hitherto mainly been of interest in connective tissue diseases. The similarities are striking, but there are also differences. We can agree with previous authors that leprosy may be a "unique immuno-pathological disease model" (9), but this model may be misleading.

However, we feel that we should pay more attention to what is going on immunologically in the diseased tissue than what may happen to be found in the serum. We should, like war correspondents, go to the actual theater of war, and not only listen to the rumors.

SUMMARY

Fifty-one biopsies from 24 patients suffering from leprosy have been examined for the presence of anti-γ-globulin activity by the mixed agglutination technique. In 31 biopsies anti-γ-globulin activity was found in the affected tissue, and the activity disappeared when the inflammation regressed. There was a striking correlation between the clinical, histologic and immunologic activities.

Positive reactions were found in lepromas and erythema nodosum leprosum. One leproma from a dimorphous case gave positive mixed agglutination, but biopsies from tuberculoid lesions gave no reaction. These latter lesions, however, were few and clinically and histologically inactive. Biopsies from positive lepromin reactions in patients with tuberculoid leprosy showed granulomas with anti-γ-globulin activity.

The anti-γ-globulin activity in the lesion was apparently connected with γG, γM and γA which showed the characteristics of rheumatoid factor. However, there was no correlation between the occurrence of anti-γ-globulin in the lesions and the presence of rheumatoid factors in serum. In some lesions the component C1q was responsible for the main activity.

A positive correlation was found between the occurrence of anti-γ-globulins and the presence of *M. leprae* in the lesions. Immunoglobulins in complex with antigens may therefore be responsible for the binding and production of anti-γ-globulin.

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