

Immunofluorescence Studies on Renal Tissue, Tonsils, Adenoids, Nasal Polyps, and Skin of Atopic and Nonatopic Patients, with Special Reference To IgE

THEA M. FELTKAMP-VROOM, P. J. STALLMAN,
R. C. AALBERSE, AND
EVELIENE E. REERINK-BRONGERS

*Central Laboratory of the Netherlands Red Cross Blood Transfusion Service
and Laboratory of Experimental and Clinical Immunology,
University of Amsterdam, Amsterdam, The Netherlands*

Received December 23, 1974

Immunofluorescence studies were performed on tissues from atopic and nonatopic patients. When these tissues were tested with a fluorescein-labeled sheep anti-human-IgE serum the cytoplasm of plasma cells and the outer zone of mast cells showed fluorescence. In a series of 196 patients a correlation was found between the clinical diagnosis and atopic disease and bright mast cell membrane IgE fluorescence. The intensity of the mast cell IgE fluorescence did not appear to correlate with the IgE level in the serum. Intraepithelial mast cells were incidentally observed in tonsils, adenoids, and nasal polyps. IgE was not found in glomeruli or vessels in renal tissue from 192 patients, among whom were patients with malignant nephrosclerosis and the nephrotic syndrome with minimal lesions.

A fifth class of human immunoglobulin, called IgE, has been described by Ishizaka *et al.* (1) and Johansson and Bennich (2). With the immunofluorescence technique it was demonstrated that IgE is produced by plasma cells in tonsils, adenoid tissue, bronchial and peritoneal lymph nodes, Peyer's patches, and the mucosal tissue of the nose, respiratory system, stomach, small intestine, and rectum (3,4). IgE could also be demonstrated on human basophils by autoradiography (5), by inhibition of rosette formation with specific anti-IgE serum (6) and with the immunoferritin technique (7). Gerber *et al.*, in an immunofluorescence study, reported the presence of IgE in asthmatic lungs (8), in lesions of malignant nephrosclerosis (9), and in the glomeruli of patients with the nephrotic syndrome (10). McPhaul *et al.* (11) reported that IgE was present in glomerular deposits in immune-mediated glomerulonephritis and Provost and Tomasi (12) were able to demonstrate IgE in dermal tissue of bullous pemphigoid patients. Cameron (13) mentioned the absence of IgE in glomeruli of an atopic patient with the nephrotic syndrome. Roy *et al.* (14) could not demonstrate IgE in glomeruli of 19 patients with the idiopathic nephrotic syndrome, nor in kidney biopsy specimens from 17 patients with a variety of renal diseases. None of these investigators described fluorescence of mast cells with the applied anti-IgE sera. In the present study the immunofluorescence technique was used to investigate the presence of IgE in human tissues, especially in renal tissues.

TABLE 1
DISTRIBUTION OF AGE AND SEX OF 192 PATIENTS WHOSE RENAL TISSUE WAS STUDIED

Sex	Age (yr)						
	0-9	10-19	20-29	30-39	40-49	50-59	60-69
F	14(8) ^a	6(2)	16(4)	13(2)	18(7)	7(2)	6
M	27(13)	12(5)	24(4)	16(6)	13(4)	9(1)	8

^a The number of sera investigated for IgE is shown in parentheses.

MATERIALS AND METHODS

Renal Tissues

Renal tissue specimens were obtained from 192 patients by biopsy (107 cases), by nephrectomy (63 cases) and by autopsy (22 cases). Distribution of age and sex of these patients is shown in Table 1. The patients were suffering from various renal diseases, e.g., malignant hypertension (six patients), acute/subacute/chronic glomerulonephritis (18 patients), chronic renal insufficiency (41 patients), nephrotic syndrome (32 patients), rapidly progressive glomerulonephritis (three patients), Goodpasture's syndrome (two patients), SLE (12 patients). Six patients did not suffer from renal disease. Four of the 192 patients showed a nephrotic syndrome in combination with atopic disease (Table 2, patients 1-4). As far as could be ascertained from the case histories, no clinical signs of atopy were present in the other 188 patients.

Skin Tissues

Skin biopsy specimens from eight of the patients with renal disease were studied. Among these were the four atopic patients with the nephrotic syndrome (Table 2).

TABLE 2
SEX, AGE, SERUM IgE LEVEL, CLINICAL DIAGNOSIS, AND MAST CELL
IgE FLUORESCENCE OF THE ATOPIC PATIENTS

Patient	Sex	Age (yr)	IgE level (U/ml)	Clinical diagnosis	Mast cell IgE fluorescence
1	M	5	>2000	Bronchial asthma + NS ^a	Bright
2	F	9	>2000	Bronchial asthma + NS	Bright
3	M	9	< 400	Hay fever + NS	Bright
4	M	29	< 400	Bronchial asthma + NS	Weak
5	M	2	400-2000	Bronchial asthma	Bright
6	F	27	>2000	Hay fever	Bright

^a NS = nephrotic syndrome.

Tonsils, Adenoids, and Nasal Polyp

Sections of tonsils and adenoids from one male and of the nasal polyp from one female atopic patient (Table 2, patients 5 and 6), and of the tonsils from two nonatopic children were used for the testing of the anti-IgE serum.

Semiquantitative Estimation of IgE in Serum

The IgE level in the serum of 63 patients (59 patients whose renal tissue was studied including the four atopic patients, and the four patients whose tonsils, adenoids, and nasal polyp were studied) was estimated semiquantitatively by double diffusion in agar (Table 1). The localization and intensity of the precipitation lines were compared with those resulting from the reaction between the anti-IgE serum and samples with a known IgE content (i.e., dilutions in normal human serum (NHS) of a serum containing ca. 60,000 IU/ml, referred to the WHO standard 68/341) (Fig. 1). By this method a rough estimation could be made of an IgE level of less than ca. 400 IU/ml (normal), about ca. 400–2000 IU/ml (slightly elevated) or more than 2000 IU/ml (definitely elevated).

Antisera

Rabbit antisera against human IgG, IgM, IgA, complement, and fibrinogen were prepared by Miss M. van der Giessen from the department of immunochemistry of our institute. They were tested for specificity with immunoelectrophoresis, agar double diffusion, and tanned red cell agglutination techniques.

The anti-IgE serum was produced by immunizing sheep with fractions rich in polyclonal IgE, isolated according to Ishizaka (1) from serum of two patients with a microfilarial and a schistosomal infection, respectively. The antiserum was made monospecific for precipitation reactions by stepwise absorption with NHS. In Fig. 2 it is shown that the anti-IgE (aIgE) gave a reaction of identity with the

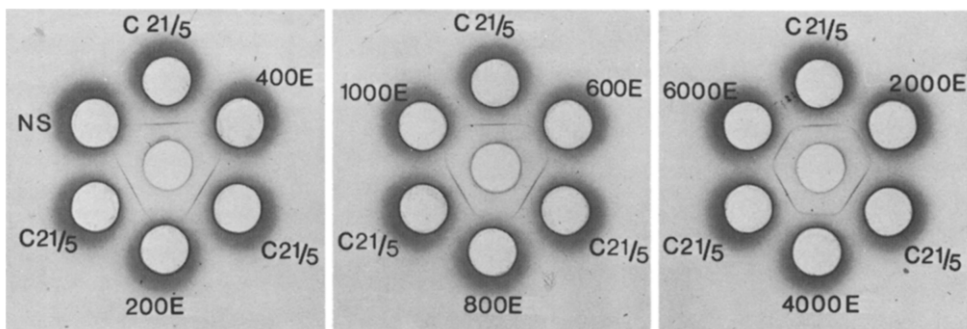


FIG. 1. Semiquantitative estimation of serum IgE level. C 21/5 means the reference IgE serum diluted 1:5 (IgE level 12,000 U/ml).

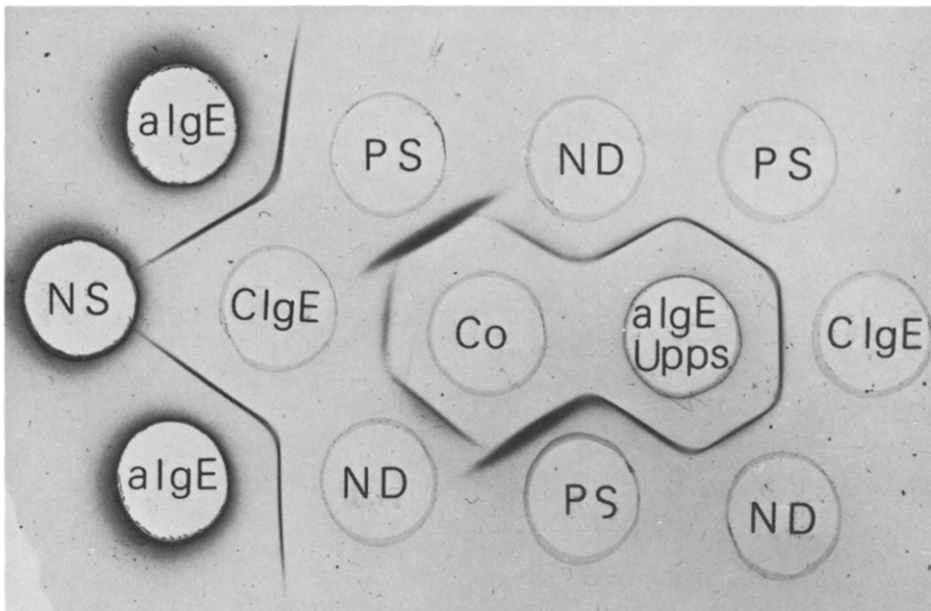


FIG. 2. Specificity of anti-IgE. PS, IgE myeloma PS; ND, IgE myeloma ND; algE, anti-IgE Amsterdam; algE Upps, anti-IgE Uppsala; Co, fluorescent anti-IgE; C IgE, polyclonal IgE fraction (see text). Another experimental fluorescent anti-IgE serum (Co) produced a precipitation line with PS, spurring over ND and C IgE. As tonsils treated with Co showed fluorescent structures that did not disappear after absorption of Co with C IgE, this antiserum was discarded from further studies. An anti-IgE serum prepared by Dr. S. G. O. Johansson (algE Upps) also gave a reaction of identity with ND, PS, and C IgE.

IgE fraction from patient C (C IgE) and the IgE myeloma proteins ND¹ (2) and PS¹ (15) and no reaction with NHS.

Conjugation of Antisera

Horse anti-rabbit immunoglobulin serum was conjugated to fluorescein-isothiocyanate (FITC) according to The and Feltkamp (16). This fluorescent anti-rabbit Ig serum was characterized by a protein concentration of 12 mg/ml, an agar block titration titer (17) of 1:32 and a molar F/P ratio of 1:8, when freed from proteins with F/P ratio of <1 and >4. A final serum dilution of 1:70 in phosphate-buffered saline (PBS) (0.01 M, pH 7.2) was used in this study. The anti-IgE was conjugated with fluorescein-isothiocyanate, and the direct immunofluorescence technique was used for the demonstration of IgE in tissues. Fluorescein was conjugated to the immunoglobulin fraction of the unabsorbed anti-IgE. After Sephadex G 25 gel filtration the conjugate was absorbed by

¹ ND and PS were kindly provided by Dr. H. Bennich and Drs. S. Kochwa and K. Ishizaka, respectively.

stepwise addition of pooled NHS in order to make the antiserum monospecific in precipitation reactions. The conjugated material was subsequently freed from proteins with F/P ratios of <1 and >4 , by which a mean molar ratio of fluorescein to sheep IgG of 2.8 was obtained. This conjugate was further absorbed with pooled NHS until tanned red cells coated with IgA, IgD, IgG, IgM, and κ and λ light chains, albumin, fibrinogen, transferrin, and the complement factors C3d, C3c, C4 were no longer agglutinated. The specificity of the conjugate was ascertained by fluorescence inhibition using tonsil sections as substrate. The fluorescence was abolished by absorption with myeloma PS or by IgE-rich serum C, but not by NHS.

In this study the final FlaIgE preparation was used, diluted 1:30 in PBS.

Light Microscopy

Renal tissue specimens were fixed in Tellyesniczky's fluid, dehydrated, and embedded in paraplast. One- to two-micron sections were stained with periodic acid-Schiff (PAS), toluidine blue (TB), Azan, Jones' periodic acid silver methenamine (PASM), Congo red, van Gieson elastin (vGE), and phosphotungstic acid hematoxylin (PTAH). When enough tissue was available portions of skin tissue were fixed in buffered formalin (pH 7.2), dehydrated, and embedded in paraplast. Four-micron sections were stained with PAS, TB, and vGE. When only minute skin biopsy specimens were obtained, the specimens were snap frozen in liquid nitrogen, and cryostat sections were stained with PAS, TB, and vGE.

Tonsils, adenoids, and nasal polyp specimens were fixed in buffered formalin (pH 7.2), dehydrated, and embedded in paraplast. Two- to four-micron sections were stained with hematoxylin and azophloxin (HA), May-Grünwald Giemsa (MGG), and TB. For TB staining toluidine blue solutions of 0.1% and 0.25%, both with either pH 2.8 or pH 5.8 were used. When cells were observed with metachromatically staining granules, which were uniformly distributed in the cytoplasm, these were identified as mast cells.

Immunofluorescence Studies

All tissue specimens were snap-frozen in liquid nitrogen. When only minute tissue specimens were available, the freezing technique was used as previously described (18). The tissues were stored in liquid nitrogen until use. Two- to four-micron sections were cut in a cryostat at -20°C , air-dried, fixed in acetone (10 min), and stained with antisera. For the demonstration of IgG, IgM, IgA, complement, and fibrinogen, the indirect immunofluorescence technique was used with the rabbit antisera as the first and the fluorescent horse anti-rabbit Ig as the second layer (19). In control experiments rabbit serum was used as first layer.

For the detection of IgE, the direct immunofluorescence technique was used. Frozen sections were air-dried, fixed in acetone, washed with PBS for 30 min at 20°C , and incubated with FlaIgE for 60 min at 20°C . Sections were again washed in PBS for 30 min and mounted in a 1:1 glycerin PBS solution. During the testing of the FlaIgE, frozen sections were used, both fixed in acetone and

unfixed. When it became evident that no difference could be found between fixed and unfixed sections, only fixed sections henceforth were used. For the investigation of mast cells, acetone-fixed cryostat sections of tonsil, nasal polyp, or adenoid were stained with TB, photographed, and after washing in PBS for 10 min restained with FlalIgE. The same fields as before were photographed again.

Slides were read in a Leitz Orthoplan fluorescence microscope equipped with incident illumination for excitation as described by Ploem (20) and Hijmans *et al.* (21). A Xenon lamp (Osram XBO 150W) was used for excitation. All fluorescence micrographs were made on Ansco 500.

RESULTS

Light Microscopy

Renal tissues. The renal tissues showed a variety of histological pictures, e.g., normal renal histology (13 cases), slight renal lesions (21 cases), various kinds of glomerulonephritis (64 cases), amyloidosis (4 cases), malignant nephrosclerosis (6 cases), Fabry's disease (1 case), and very advanced renal lesions (41 cases).

From the atopic patients listed in Table 2, patients 1-3 showed minimal glomerular lesions, and patient 4 membranous glomerulonephritis.

With respect to mast cells, it was observed that in renal tissue without interstitial infiltration and/or fibrosing lesions, mast cells could only be found around interlobular, arcuate, and larger vessels. However, in fibrosing areas and sometimes in inflammatory foci an increase in the number of mast cells could be observed. This implies that mast cells were found more abundantly in interstitial nephritis, malignant nephrosclerosis, and glomerulonephritis, all in combination with fibrosing lesions, than in kidneys with only minimal lesions of the glomeruli.

Skin tissues. In the skin sections mast cells were found predominantly around vessels.

Tonsils, adenoids, and nasal polyp. Tonsils and adenoids of patient 5 in Table 2 showed many large lymph follicles with germinal centers. Under the epithelial layer and between the lymph follicles plasma cells, eosinophils, and mast cells were easily found. Sometimes a mast cell was found lying as an elongated structure between the basal cells of the stratified squamous epithelium. The nasal polyp of patient 6 in Table 2 consisted of loose stroma lined with pseudostratified columnar epithelium with an occasional intraepithelial elongated mast cell. Nodular and diffuse lymphoid cell infiltrates were present as well as large rounded or perivascularly lying elongated mast cells, plasma cells, and eosinophils. The tonsils of the two nonatopic children showed fewer eosinophils than those of the atopic patient, but otherwise the histological picture was identical. Intraepithelial mast cells were also present in these tonsils.

Immunofluorescence

Nasal polyp, adenoids, and tonsils. Since mast cells were easily found in the nasal polyp, adenoids, and tonsils, and since it was expected that, on the analogy

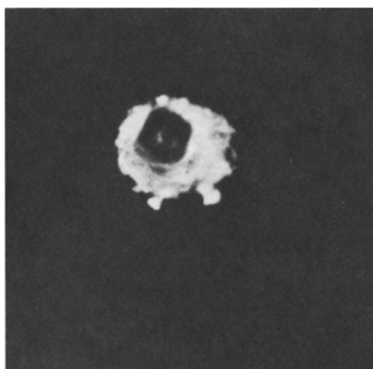


FIG. 3. Plasma cell in the tonsil of an atopic patient. aIgE(500 $\times$).

of human basophils, IgE might be found on mast cells, these tissues were the first to be studied with FlalIgE. Sections of the tissues incubated with FlalIgE showed two fluorescence patterns: bright fluorescence of the cytoplasm of plasma cells (Fig. 3), and a delicate fluorescence of the outer zone of mast cells (Fig. 4). Sometimes the delicate fluorescence of mast cells appeared to be linear, like a cell membrane fluorescence; at other times, however, there appeared to be also a faint mesh-like fluorescence just inside the fluorescent cell membrane (Fig. 5). It was not possible to interpret this mesh-like fluorescence: whether it was caused by IgE fluorescence on a tangentially cut mast cell membrane or by the presence of IgE in the outer zone of the mast cell cytoplasm. The same holds true for mast cells lying between the epithelial lining cells (Figs. 6,7). By re-staining toluidine blue-stained sections with FlalIgE, it was found that every mast cell present was marked by weak fluorescence. Concerning the intensity of the IgE fluorescence of mast cells, it appeared that mast cells in tonsils, nasal polyps, and adenoids of the atopic patients revealed a brighter fluorescence than mast cells in the tonsils of the nonatopic patients. In the tonsils of the nonatopic patients, the IgE fluorescence of mast cells was very weak, and sometimes even

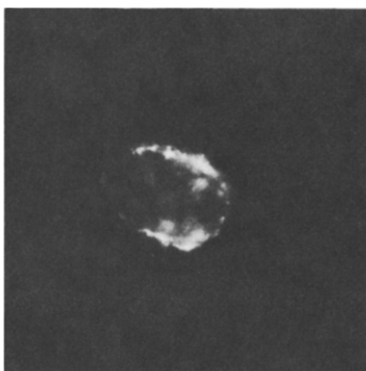


FIG. 4. Rounded mast cell in the nasal polyp of an atopic patient. aIgE(500 $\times$).

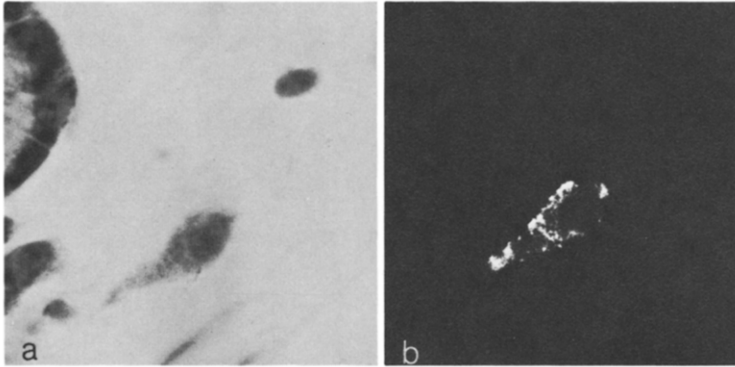


FIG. 5. a.Mast cell in the nasal polyp of an atopic patient. Toluidine blue(500 $\times$). b.Same section restained with aIgE.

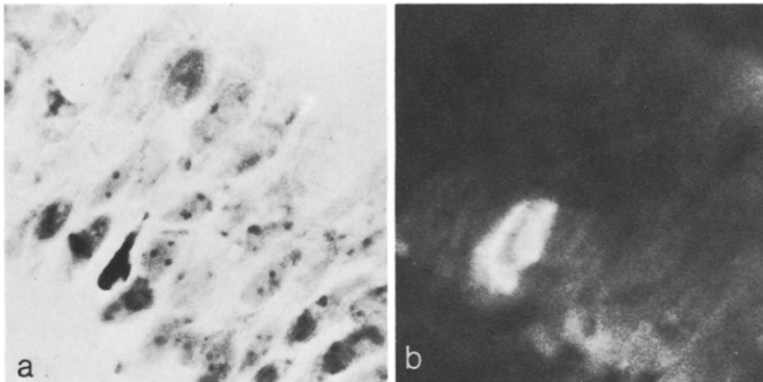


FIG. 6. a.Intraepithelial elongated mast cell in the tonsil of an atopic patient. Toluidine blue(500 $\times$). b.Same section restained with aIgE.

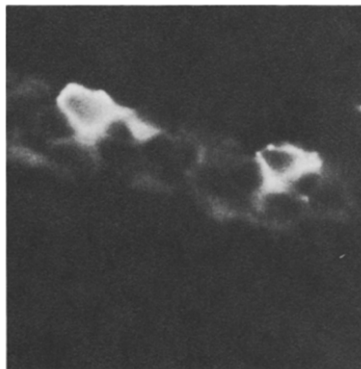


FIG. 7. Two intraepithelial mast cells in the nasal polyp of an atopic patient. aIgE(500 $\times$).

hardly detectable. Occasionally patchy IgE was observed intercellularly in germinal centers of the lymph follicles.

Specific fluorescence of eosinophils with FlIgE could not be demonstrated, but it cannot be excluded that the strong aspecific fluorescence of these cells might mask the presence of small amounts of IgE. Eosinophils could be distinguished from plasma cells and mast cells by using light microscopy, in combination with fluorescence microscopy.

IgE-containing plasma cells seemed to be present in a greater number in the tissues of the two atopic patients than in those of the nonatopic patients. Other plasma cells in tonsils, adenoids, and nasal polyp contained either IgA, IgM, or IgG. These immunoglobulins were also found between the cells of the germinal centers.

Renal tissues. In 41 out of the 192 samples of renal tissue, granular deposition of IgG or IgM and complement was present along the glomerular basement membrane.

The glomeruli of three patients with rapidly progressive glomerulonephritis and of the two patients with Goodpasture's syndrome showed a linear pattern of IgG and complement deposition. The six patients with malignant nephrosclerosis showed heavy IgM, IgG, and complement deposition in small arteries, arterioles, and glomeruli. In 17 out of the 21 cases with slight renal lesions, the immunofluorescence findings were negative. These patients were diagnosed as having minimal lesion disease (idiopathic nephrotic syndrome). Of the remaining four cases, three showed fine granular IgG and complement deposition and one mesangial IgA and complement deposition. In none of the 192 patients could IgE be demonstrated, either in glomeruli or in vessel walls. As in tonsils, adenoids, and the nasal polyp, IgE was found only on mast cells. It must be emphasized that in three atopic patients (Table 2, patients 1-3) mast cells present around renal vessels showed bright mast cell fluorescence, similar to that demonstrated in the adenoids, tonsils, and nasal polyp of two such patients (Table 2, patients 5 and 6). In another atopic patient (Table 2, patient 4) with

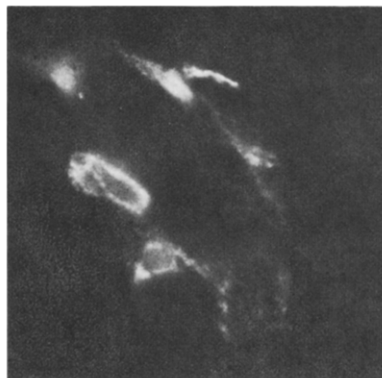


FIG. 8. Perivascular mast cells in the skin of an atopic patient. aIgE(500 $\times$).

TABLE 3
IgE LEVELS IN SERA OF ATOPIC AND NONATOPIC PATIENTS

IgE in serum (U/ml)	Number of patients			Renal tissue studied		Tonsils, adenoids, or nasal polyp studied	
	Total	Atopic	Nonatopic	Atopic	Nonatopic	Atopic	Nonatopic
0-ca. 400	51	2 (1) ^a	49	2 (1)	47	0	2
ca. 400-ca. 2000	6	1 (1)	5	0	5	1 (1)	0
>2000	6	3 (3)	3	2 (2)	3	1 (1)	0
Total number	63	6	57	4	55	2	2

^a The number of patients in whose tissues a bright mast cell fluorescence was observed is shown in parentheses.

bronchial asthma (for 25 yr) and membranous glomerulonephritis, however, only weak mast cell IgE fluorescence was observed.

Skin tissues. The skin tissue specimens of the three atopic patients with the nephrotic syndrome (Table 2, patients 1-3) showed bright mast cell IgE fluorescence (Fig. 8), whereas only a very weak reaction was observed in the skin specimen of the fourth atopic patient (Table 2, patient 4) and the four other patients.

IgE levels of sera. IgE levels were estimated in the sera of 63 patients (Table 3). A definitely elevated IgE level (2000 IU/ml) was found in six patients, i.e., three atopic (Table 2, patients 1, 2, and 6) and three nonatopic patients with renal disease. A slightly elevated IgE level (400-2000 IU/ml) was found in six patients, i.e., one atopic (Table 2, patient 5) and five nonatopic patients with renal disease. The three nonatopic patients with renal disease and a definitely elevated IgE level suffered from parasitic infections (one from scabies and two from visceral worms).

Since bright mast cell fluorescence was found in five atopic patients (Table 2, patients 3, 5, and 6), while only one atopic (Table 2, patient 4), and all nonatopic patients showed a weak mast cell fluorescence, regardless of their IgE level, it is evident that, in this series of patients, a correlation exists between a bright mast cell IgE fluorescence and the diagnosis of atopic disease as based on anamnestic data.

DISCUSSION

With an anti-IgE serum conjugated to fluorescein, it has been possible to demonstrate IgE in human tissues, e.g., kidney, tonsils, adenoids, nasal polyp, and skin. It is of particular interest that IgE not only could be found in plasma cells, as already described by several investigators (3,4), but also in the outer zone of mast cells.

Ishizaka *et al.* (5, 22, 23) reported that IgE is present on human basophils and on monkey lung mast cells and that as the result of the reaction between mast

cell-bound IgE and allergen or anti-IgE, histamine and other mediators are released. The observation that IgE is present on and possibly in human mast cells, therefore, suggests that in human tissues mast cells play the same role as the human basophil and monkey mast cell.

Of interest is the finding of mast cells lying in the epithelium of adenoids, tonsils, and the nasal polyps. Zwillenberg (24) described the occurrence of intraepithelial mast cells in the portio vaginalis uteri of the human. The intraepithelial, metachromatically staining mast cells demonstrated in our study were sometimes elongated, sometimes polygonal. Since they were lying near the epithelial basement membrane, it seems likely that the mast cells move into the epithelium from the underlying tissue by ameboid movement, as described for rat mast cells (25,26). In this situation an inhaled allergen can quite easily find sensitized mast cells lying superficially along the upper respiratory tract. Our finding concerning mast cells in renal tissue confirms the data of Pavone-Macaluso (27), who stated that the normal kidney is almost devoid of mast cells, but that when inflammation and sclerosis are present, mast cells are observed. It must be emphasized that in our study, in kidneys with minimal lesions no more mast cells seemed to be present than in normal kidneys. Another interesting finding was that mast cells in five of six atopic patients showed brighter staining for IgE than those of nonatopic patients (Table 3). Therefore, the finding of bright IgE fluorescence of mast cells is highly suggestive for atopy; however, it cannot be said that weak fluorescence pleads against atopy. Unfortunately, the tissue specimens did not suffice for a study with diluted aIgE to estimate the fluorescence titer of mast cell fluorescence. Preliminary results of a study on IgE in bronchial biopsy specimens indicate a correlation between bright mast cell fluorescence (titer 1:80) and clinical diagnosis of atopy (Feltkamp-Vroom *et al.*, in preparation). In our study, elevated serum IgE levels were found in four of the six atopic patients and in eight of the 57 nonatopic patients. Three nonatopic patients with high IgE levels suffered from parasitic infections (scabies and visceral worms). It is known from studies of Johansson *et al.* (28), Hogarth-Scott *et al.* (29), Rosenberg *et al.* (30), and Spitz *et al.* (31) that patients with parasitic infections may show an elevated IgE level. One of our patients suffered from a scabies infection; further investigation is needed to determine whether this can be incriminated as a cause of increased IgE levels. The reason for the IgE elevation of the other five nonatopic patients is not clear. Our finding that bright IgE fluorescence of mast cells is related to the clinical diagnosis of atopy and not to the IgE level of the serum, is in accordance with the data of Ishizaka *et al.* (32), who demonstrated that the amount of basophil-bound IgE is not correlated with the IgE level of the serum. The results of our study on renal tissue are similar to those of Roy *et al.* (14), who did not find IgE in glomeruli and vessel walls. Hence, these authors and ourselves have been unable to confirm the reports stating that IgE is located in the walls of many arterioles in the renal tissue of patients suffering from malignant nephrosclerosis (9) or in glomeruli of patients with the nephrotic syndrome and minimal glomerular lesions (10), or in glomeruli of patients with immune-mediated glomerulonephritis (11).

Since we did not find IgE in glomeruli with granular deposits (41 cases) not even in one atopic patient with the nephrotic syndrome (Table 2, patient 4), we think that it is not likely that IgE is a constituent of immune complexes, involved in the pathogenesis of glomerulonephritis. Reeves *et al.* (33), have suggested that there are cells in the kidney that are different in appearance from mast cells, but which may be the site of fixation of IgE; however, we have found no cells other than mast cells with cell-bound IgE.

ACKNOWLEDGMENTS

We thank Miss M. v.d. Veer from the Rijksinstituut voor de Volksgezondheid, Bilthoven, Mr. M. J. O. Calliauw, Miss J. B. van Heertum, Mr. H. J. Ruis, Mrs. C. B. de Graaff-Reitsma, and Mr. A. Rol for expert technical help and Mrs. M. van Wijck-Scholten for typing this manuscript. This study was performed with financial aid from the Dutch Kidney Foundation.

REFERENCES

1. Ishizaka, K., Ishizaka, T., and Hornbrook, M. M., *J. Immunol.* **97**, 840, 1966.
2. Johansson, S. G. O., and Bennich, H., *Immunology* **13**, 381, 1967.
3. Callera, M. L., Candemi, J. J., Ishizaka, K., and Vaughan, J. H., *Clin. Res.* **18**, 423 (abstr.), 1970.
4. Tada, T., and Ishizaka, K., *J. Immunol.* **104**, 337, 1970.
5. Ishizaka, K., Tomioka, H., and Ishizaka, T., *J. Immunol.* **105**, 1459, 1970.
6. Wilson, A. B., Marchand, R. M., Wilson, D. V., Devey, M., and Coombs, R. R. A., *Int. Arch. Allergy* **42**, 668, 1972.
7. Sullivan, A. L., Grimley, P. M., and Metzger, H., *J. Exp. Med.* **134**, 1403, 1971.
8. Gerber, M. A., Paronetto, F., and Kochwa, S., *Amer. J. Pathol.* **62**, 339, 1971.
9. Gerber, M. A., and Paronetto, F., *Amer. J. Pathol.* **65**, 535, 1971.
10. Gerber, M. A., and Paronetto, F., *Lancet* **1**, 1097, 1971.
11. McPhaul, J. J., Jr., Newcomb, R. W., Mullins, J. D., Thompson, A. L., Jr., Lordon, R. E., and Rogers, Ph. W., *Kidney Int.* **5**, 292, 1974.
12. Provost, F. T., and Tomasi, T. B., Jr., *Clin. Exp. Immunol.* **18**, 193, 1974.
13. Cameron, J. S., *Brit. Med. J.* **4**, 160, 1972.
14. Roy, L. P., Westberg, H. G., and Michael, A. F., *Clin. Exp. Immunol.* **13**, 553, 1973.
15. Ogawa, M., Kochwa, S., Smith, C., Ishizaka, K., and McIntire, O. R., *N. Engl. J. Med.* **281**, 1217, 1969.
16. The, T. H., and Feltkamp, T. E. W., *Immunology* **18**, 875, 1970.
17. Feltkamp, T. E. W., In "Standardization in Immunofluorescence" (E. J. Holborow, Ed.), pp. 189-191, Blackwell, Oxford, 1970.
18. Feltkamp-Vroom, Th. M., and Boode, J. H. M., *J. Clin. Pathol.* **23**, 188, 1970.
19. Roos, O. M., Feltkamp-Vroom, Th. M., and Helder, A. W., *J. Pathol.*, in press.
20. Ploem, J. S., *Z. wiss. Mikr.* **68**, 129, 1967.
21. Hijmans, W., Schuit, H. R. E., Jongsma, A. P. M., and Ploem, J. S., In "Standardization in Immunofluorescence" (E. J. Holborow, Ed.), pp. 193-202, Blackwell Sci. Publ., Oxford, 1970.
22. Ishizaka, T., Ishizaka, K., and Tomioka, H., *J. Immunol.* **108**, 513, 1972.
23. Ishizaka, T., De Bernarda, R., Tomioka, H., Lichtenstein, L. M., and Ishizaka, K., *J. Immunol.* **108**, 1000, 1972.
24. Zwillenberg, L. O., *Nature (London)* **18**, 1343, 1958.
25. Cambel, P., Conroy, C. E., and Squires, J. T., *Science* **115**, 373, 1952.
26. Selye, H., Gabbiani, G., and Nielsen, K., *Proc. Soc. Exp. Biol. Med.* **112**, 460, 1963.
27. Pavone-Malcaluso, M., *Acta Pathol. Microbiol. Scand.* **50**, 337, 1960.
28. Johansson, S. G. O., Melbin, T., and Vahlquist, B., *Lancet* **1**, 1118, 1968.
29. Hogarth-Scott, R. S., Johansson, S. G. O., and Bennich, H., *Clin. Exp. Immunol.* **5**, 619, 1969.

30. Rosenberg, E. B., Whalen, G. E., Bennich, H., and Johansson, S. G. O., *N. Engl. J. Med.* **283**, 1148, 1970.
31. Spitz, E., Gelfand, E. W., Sheffer, A. L., and Austen, K. F., *J. Allergy Clin. Immunol.* **49**, 337, 1972.
32. Ishizaka, T., Soto, C. S., and Ishizaka, K., *J. Immunol.* **111**, 500, 1973.
33. Reeves, W. G., Cameron, J. S., and Ogg, C. S., *Lancet* **1**, 1299, 1971.