Immunoglobulin A Nephropathy

QUANTITATIVE IMMUNOHISTOMORPHOMETRY OF THE TONSILLAR PLASMA CELLS EVIDENCES AN INVERSION OF THE IMMUNOGLOBULIN A VERSUS IMMUNOGLOBULIN G SECRETING CELL BALANCE

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ABSTRACT Primary IgA nephropathy (Berger's disease) is characterized by renal deposits of IgA, the origin of which is still unknown. However, several clinical and biological findings suggest that these immunoglobulins might have a mucosal origin, and that such patients should present mucosal abnormalities.

This paper reports the results of the immunohistomorphometrical analysis of tonsillar plasma cells from seven patients suffering from Berger's disease and seven controls also with recurrent tonsillitis. IgG, IgA, and IgM-secreting cells were enumerated after immunofluorescent staining of serial frozen-cut sections from 20 tonsils. In controls, a predominance of the IgG-secreting population, similar to this reported in the literature was observed (65% of IgG secreting cells and 29% of IgA plasma cells), while an inversion in the patients' plasma cells percentages was evidenced (IgG:37%, IgA:56%).

This increment in the IgA population was paralleled by an augmentation of the number of dimeric IgA-secreting cells (75% of IgA plasma cells), stained both for cytoplasmic IgA and J chain. In controls, the latter cells were in similar proportions as previously reported by others (45% of IgA plasma cells).

These results demonstrate an imbalance in the IgA-producing system of patients with Berger's disease, which is in keeping with the hypothesis favoring a mucosal origin for the mesangial IgA present in their kidneys.

INTRODUCTION

Since its first description by Berger and Hinglais in 1968 (1), primary IgA nephropathy has been an etiopathological challenge for nephrologists and immunologists. At the moment, after large numbers of various explorations have been performed both on kidneys and in serum from patients with Berger's disease, a series of well established facts seems to lead towards a better understanding of what happens in this peculiar disease. First, the presence of C3, C9, and occasionally factor B and properdin in the mesangial deposits (2–6), together with the pathognomonic IgA, allows to classify Berger's disease as an "immune complex nephritis" (7). This is in keeping with the reported finding of IgA-containing circulating immune complexes in such patients' serum (8–10). Second, serological studies have often evidenced high levels of circulating IgA (3, 5, 8–11), independent of the presence of circulating immune complexes, suggesting the existence of some primary abnormality. An imbalance of the IgA system might take place at two levels, according to the systemic or mucosal origin of these immunoglobulins. Frequent observation of recurrent infections of the upper respiratory tract, often occurring prior to renal manifestations, represents the third piece of evidence displaying an etiopathological pattern for Berger's disease.

This hypothesis figures that mesangial IgA may have a mucosal origin, and recently received some support with the demonstration of these IgA polymeric nature (12, 13). This theory takes account of the serological abnormalities aforementioned, but still needs to be thoroughly supported by pieces of evidence.

Tonsillectomy and dental treatment have been reported as efficient means to prevent an early onset of renal failure (14, 15). We have investigated the plasma cell populations of tonsils from patients suffering from primary IgA nephropathy who underwent tonsillectomy, providing us with a convenient way of studying a part of their secretory immune system.

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Our study evidences an inversion in the population of IgA vs. IgG plasma cells in tonsils from Berger's disease patients, as compared to a series of controls.

METHODS

Patients with IgA nephropathy

Seven patients (two females, five males) suffering from IgA nephropathy were included in this study. The average age was 28 yr (range, 17–44). Berger's disease was suggested by clinical and biological findings, and confirmed by immunological study of kidney biopsies. All diagnoses were established in our laboratory, by evidence of mesangial deposits, labeled by fluorescent monospecific antisera to human alpha chains, C3 and C9 (Behring AG, Marburg, FRG). All patients had elevated levels of serum IgA as determined by laser immunonephelometry (mean value 344.5 mg/100 ml, range 253–480 mg/100 ml; reference interval in controls, 130–300 mg/100 ml).

Controls

The control series included seven age-matched males who had undergone tonsillectomy as treatment for recurrent infections of the upper respiratory tract, therefore presenting with the same tonsillar background as the patients. Five were free from renal troubles. Two had minimal signs of renal lesion (mild proteinuria and microscopic hematuria), but immunohistological study of kidney biopsies failed to characterize Berger's disease: one was free from deposits of any kind, the other contained faintly labeled deposits of C3 in the mesangial area, similar to those described by Orfila et al. (16).

Tissue specimens

A total number of 20 palatine tonsil specimens were studied, since both right and left tonsils were obtained from five patients and one control. They were removed surgically, and each sample was snap-frozen in liquid nitrogen immediately after removal, then kept at −75°C until studied.

Immunofluorescence techniques

Serial tissue sections were frozen-cut at 3.5 μm, the plane of the section being oriented normally to the tonsil's epithelial surface. The first section was stained with toluidine blue (as currently done for frozen-cut material) for orientative histologic analysis. Nine consecutive sections were then collected on clean glass slides, and washed 5 min in phosphate-buffered saline (PBS) at room temperature. Three sections were stained with each of the following fluorescein-conjugated antisera:anti-human gamma, alpha, and mu heavy chains (Behring). These reagents had previously been tested in immunofluorescence on a large number of other human tissues to assess the absence of nonspecific labeling of epithelial or other unconcerned cells. Their monospecificity was also checked by immunofluorescence on bone marrow smears of immunochemically defined myelomas and frozen-cut sections of plasmacytomas. This monospecificity was further confirmed by "blocking experiments" performed on tonsillar sections after incubation of the antiserum with purified aggregated immunoglobulins.

The absence of cross-reactivity was checked by double-labeling some tonsil sections with fluorescein-conjugated anti-gamma chain serum and rhodamine-conjugated anti-alpha chain serum (Cappell Laboratories, Cochranville, PA). The latter specificity had been tested both in immunofluorescence and immunodiffusion.

All immunofluorescence tests were performed at room temperature by incubation of the sections with a proper dilution of the adequate antiserum in PBS, in a moist chamber for 30 min. The sections were then mounted in a glycerol/saline mixture (9/1 vol/vol) under a coverslip. All slides were examined in a blind fashion by two different people.

Identification of J chain-producing plasma cells

To evaluate the number of IgA-secreting plasma cells also producing J chain, a technique adapted from this described by Brandtzaeg et al. (17) was used. Briefly, two sections from each tonsil were collected on a clean glass slide and washed in PBS for 5 min at room temperature. The slides were then placed in cold 95° ethanol and fixed at 4°C for 5 min. After another wash in PBS at room temperature the sections were submitted to denaturation in 6 M urea, pH 3.5, for 1 h at 4°C. This treatment was followed by three washes in PBS (5 min, room temperature) to remove all urea from the sections. Three periods of incubation followed, with intervals of three washes in PBS. They were respectively performed with rabbit anti-human J chain serum (Nordic Laboratories, Oslo, Norway) fluorescein-conjugated sheep anti-rabbit IgG serum (Institut Pasteur, Paris, France), and rhodamine-conjugated anti-alpha chains serum.

A series of immunofluorescence and immunochemical tests had assessed the monospecificity of the anti-rabbit IgG serum and of the anti-J chain serum. The latter was further carefully checked as follows: (*a*) It provided one single peak in double bidimensional electrophoresis against human dimeric IgA purified from colostrum (12), mildly reduced with thioerythrytol, submitted to electrophoresis with sodium dodecyl sulphate in polyacrylamide gel, and submitted to a cross electrophoresis against an agarose gel containing the anti-J chain serum. (*b*) J chain labeling was completely "blocked" after incubation of the antiserum with a purified preparation of human J chain checked in polyacrylamide electrophoresis. (*c*) J chain labeling was not modified after absorption of the antiserum against an immunosorbent prepared with human monomeric serum IgA (12).

Control slides were prepared for all sections, omitting the anti-J chain serum, which displayed no green fluorescence and assessed the absence of nonspecific labeling by the fluorescein-conjugated anti-rabbit IgG serum.

Immunohistomorphometry

Quantification of IgG, IgA, and IgM-producing cells. All sections studied contained the four tonsillar compartments described by Brandtzaeg et al. (18): extrafollicular area, follicle center, mantle zone, and reticular epithelium. The slides were observed with a Ploem system of epiillumination and selective interferential filters for green and red fluorescence.

Plasma cells enumerations were performed for tissue units defined by an ocular graticule. The magnification was 500, and each tissue unit therefore represented 0.05 mm^2. For reasons described in the results section, extrafollicular and reticuloepithelial areas only were recorded. For each immunoglobulin class, an average 400 tissue units were counted

per specimen. Cells secreting more than one immunoglobulin class were extremely rare.

Double labeling for J chain- and IgA-producing cells. About 200 cells were recorded on each section, and the percentage of doubly labeled cells was calculated. IgA-secreting cells and cells stained for both IgA and J chain were the only ones taken into account in this experiment. However, there were some cells labeled for J chain only, which provided an extra control of the absence of cross-reactivity between IgA and the anti-J chain serum, and were assumed to be IgM-producing cells.

Data analysis

IgG, IgA, and IgM secreting cell numbers were analyzed using a Student's *t* test, performed with the "index values" defined as the number of cells per surface unit. To obtain nonbiased statistical results, one tonsil only was taken into account for the *t* test in the six cases where both tonsils had been available. The selected specimen was chosen with a random number table.

RESULTS

Ig-producing cells in the follicles and mantle zones. All sections contained follicles stained with each antiserum, most of them producing one specific Ig class only. Their labeling was similar to that described by Brandtzaeg et al. (18), with a reticular pattern and some brightly stained immunocytes. Large variations were observed between individual samples, regarding the overall number of follicles. No statistically significant analysis could therefore be undertaken, comparing patients' and controls' follicles, although there was a large number of IgA-positive follicles in the patients (data not shown).

No more information could be drawn from a study of mantle zones plasma cells, which appeared to be very few, this observation again being in keeping with those of Korsrud and Brandtzaeg (19).

Ig-producing cells in extrafollicular and reticulo-epithelial areas. Plasma cells located in the extrafollicular and reticulo-epithelial areas were the only ones recorded, since they provided more comparable data. An identical immunohistomorphometrical study was performed for IgG-, IgA-, and IgM-producing cells in all tonsils. The whole surface of each section was scanned. In all cases, the greatest concentration of plasma cells appeared located in the extrafollicular compartment between the epithelium and the follicles, but the less superficial areas were also recorded. This observation again is similar to what has been reported by Korsrud and Brandtzaeg (19). The results obtained for each specimen (three sections studied with each antiserum) were pooled to calculate mean indexes and percentages for each Ig class. These data are presented in Table I.

TABLE I
Mean Indexes and Percentages of IgG, IgA, and IgM-secreting Tonsillar Plasma Cells in Patients and Controls

Patients							Controls						
	IgG		IgA		IgM			IgG		IgA		IgM	
No.	I	%	I	%	I	%	No.	I	%	I	%	I	%
*1(l)	2.4	18	9.6	73	1.2	9	1	12.1	61	6.5	33	1.9	6
1(r)	3.6	24	9.9	66	1.5	10							
2	4.8	48	4.4	44	0.7	8	2	11.5	72	3.5	23	0.8	5
3(l)	9.8	42	12.2	53	1.1	5	3	9.6	62	4.5	29	1.2	9
*3(r)	6.2	37	9.5	56	1.3	7							
4(l)	6.4	32	12.2	61	1.5	7	4	13.9	77	2.9	16	1.3	7
*4(r)	15	44	17.7	53	1.1	3							
5(l)	7.3	34	12.4	58	1.6	8	5	7	57	4.4	36	0.9	7
*5(r)	8.4	41	10.7	52	1.5	7							
6(l)	13	50	12.2	47	0.8	3	6(l)	14	58	8.9	37	1.2	5
*6(r)	8.8	40	10.7	49	2.4	11	*6(r)	6.7	68	2.8	29	0.3	3
7	7.4	32	13.7	60	1.9	8	7	13.4	65	6	29	1	6
Mean	7.7	37	11.3	56	1.4	7	Mean	11	65	4.9	29	1	6

After immunofluorescent staining, IgG, IgA, and IgM secreting cells were enumerated on nine serial sections from each specimen (three for each Ig class). Only plasma cells contained in the extrafollicular and reticulo-epithelial areas were evaluated. Index values (I) were calculated, representing the mean number of cells secreting one Ig class per tissue unit (0.05 mm^2). Specimens noted * were randomly selected for statistical analysis of these results (see Table II). l and r indicate that the following results are related to the left or right tonsil.

Table II gives the results considered for statistical analysis, in terms of mean indexes, percentages and standard errors for patients and controls. As mentioned previously, a t test was applied to seven results from each group, random choice providing the recorded data when two samples had been available for one individual.

Results in Table I clearly evidence much higher numbers of IgA-secreting cells than of IgG-producing cells in the patients, while the opposite is observed in the control group. Table II indicates a statistically significant difference ($P = 0.001$), which likely represents the major result of this study.

However, several other features deserve to be pointed out, such as the degree of imbalance in patients where the mean index value of the most represented Ig class (IgA) is much higher than this of controls' predominant Ig class (IgG). These indexes, respectively, are 11.27 and 9.90. This observation may be extended to the overall tonsillar plasma cells population: when the results of the three classes are pooled, the number of plasma cells per square millimeter is 316 in the control group, and 428 in the patients. These data indicate a higher density of plasma cells in patients' tonsils, and are within a normal range in controls as compared with what has been reported by Brandtzaeg et al. (18).

Presence of J chain in IgA-secreting cells. The study of doubly labeled sections of the 20 specimens allowed two kinds of observations. First, it demonstrated the presence of two types of IgA-secreting cells, depending on the concomitant presence or absence of J chain. Second, it evidenced a population of cells stained for J chain only. This has been reported previously (20), but in this case, the intensity of the labeling and the fairly small number of stained cells suggest that IgM-secreting cells were visualized in this indirect manner.

A quantitative analysis of dimeric IgA-secreting cells (defined by the concomitant presence of cytoplasmic IgA and J chain) was performed and compared with the total number of IgA plasma cells. The percentage of dimeric IgA-producing cells was calculated in each case. These data are reported in Table III. Patients and controls results were pooled in two groups. The mean percentage of dimeric IgA-secreting cells in controls (45%) is similar to this reported by others (19), but is much higher in patients (75%).

A t test was applied to these data, using the same randomly chosen specimens as previously. Although this led to slightly different mean percentages (42.2 and 76, respectively), the high significance of this results is expressed in a P value of 0.001.

It should finally be mentioned that this increment of the number of dimeric IgA-secreting cells parallels the global elevation of tonsillar IgA plasma cells in patients, suggesting the proliferation of one or several dimeric IgA-producing clones.

DISCUSSION

The results presented in this paper are, to our knowledge, the first demonstration of significant mucosal differences between Berger's disease patients and controls.

It shows, in seven patients, a significant increment of the tonsillar dimeric IgA-secreting plasma cell population, associated to a decrease in the IgG-producing plasma cell population.

TABLE II

Statistical Analysis of the Indexes Given in Table I

	IgG		IgA		IgM	
	Mean	SE	Mean	SE	Mean	SE
Patients	7.56	3.92	10.90	4.08	1.43	0.55
Controls	10.59	2.92	4.38	1.46	0.95	0.33
Student's t *	1.64		3.98		1.98	
	NS		*		NS	

Student's t test was applied to mean index values of seven patients and seven controls selected by random choice. Comparison between patients and controls is not significant (NS) for IgG and IgM plasma cells, but highly significant (*) ($P = 0.001$) for IgA plasma cells, which are much more numerous in patients.

TABLE III

Percentages of Dimeric IgA-secreting Cells among Tonsillar IgA Plasma Cells from Patients and Controls

Patients		Controls	
No.	%	No.	%
1(l)	73	1	50
1(r)	77		
2	76	2	32
3(l)	69	3	37
3(r)	80		
4(l)	81	4	41
4(r)	78		
5(l)	77	5	52
5(r)	61		
6(l)	72	6(l)	63
6(r)	72	6(r)	43
7	85	7	42

Dimeric IgA-secreting cells were defined as doubly labeled plasma cells after immunofluorescent staining with a rabbit anti-human J chain plus fluorescein-conjugated anti-rabbit serum, and rhodamine-conjugated anti-human alpha chain serum. An average number of 200 cells was enumerated for each section.

These results are strongly supported by the similarity between the data obtained in our control series and those reported by others in this field. There is indeed a great homogeneity in the literature's results, reporting the constant predominence of IgG plasma cells in normal tonsils as well as in patients with recurrent tonsillitis. These studies have been performed in immunofluorescence (21–24, 25), immunoperoxydase staining (26), or on suspensions of tonsillar lymphoid cells (26). In recent papers, Brandtzaeg reports thorough quantitative studies performed on palatine and adenoid tonsils in various age groups (18) or clinical conditions (19, 27), trying to avoid pitfalls eventually unnoticed in previous studies. He recommends for instance to use washed tonsil sections, which provide a better visualization of plasma cells, and emphasize the homogeneity of previously reported values by studying selected control groups where he observes a similar partition of plasma cells as in patients with recurrent tonsilitis (19). The values we recorded in our control subjects are in keeping with these recently published results. When the data reported by Brandtzaeg and Korsrud (19) are recalculated (they recorded IgD-secreting plasma cells, while we did not) and compared with ours, the differences appear very insignificant: IgG: 65.5 and 64.7%, IgA: 30.1 and 28.8%, IgM: 4.4 and 6.5%.

These observations enhance the significance of the abnormalities we noticed in Berger's disease patients. If needed, further support could be found in Surjan and Brandtzaeg results evidencing a decrease in the tonsillar plasma cell population in elder subjects (18), and in patients with recurrent tonsillitis (27). We obtained elevated values in our patients who both belong to adulthood and suffer from recurrent infectious episodes of the upper respiratory tract, including tonsillitis.

Some basic abnormality, therefore, seems to be present in these patients' tonsils, the origin of which remains to be demonstrated. A genetic background cannot be excluded, particularly since no specific infectious agent has been reported responsible for these patients' tonsillitis. This suggests an abnormal response to common germs, which might happen because of a B cell dysfunction or, at a different level, imply an imbalance of T cells regulatory subsets, or result from a dysfunction of "switch cells" preferentially leading to an IgA-type response (28).

As far as Berger's disease etiopathological mechanisms are concerned, this certainly favors the hypothesis of a mucosal origin for mesangial IgA. The excess of dimeric IgA produced as a consequence to tonsillar infection might reach the blood, possibly induce the formation of immune complexes eventually depositing in the mesangium for some still unknown reason.

In any case, these results provide a theoretical support to the therapeutic tonsillectomy proposed by some authors (14, 15). Empirical reasons only had been advanced so far, but there seems to be some good to expect from the removal of a dysfunctioning tissue, although it certainly is not the only one implied.

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