Diminished Synthesis of Immunoglobulin by Peripheral Lymphocytes of Patients with Idiopathic Membranous Glomerulonephropathy

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ABSTRACT Some studies of animal models of serum-sickness nephritis have shown that the lesions of membranous nephropathy develop in animals exhibiting a poor antibody response to the administered antigen (if given in constant amounts). It is postulated that patients with idiopathic membranous nephropathy may share a similar characteristic, namely, a diminished capacity to produce sufficient amounts of antibody. To test this hypothesis, we examined the ability of lymphocytes isolated from 11 patients with this disorder to produce immunoglobulin (Ig)G and IgM on stimulation with a polyclonal B-cell activator, pokeweed mitogen. The peripheral blood lymphocytes (2×10^6 cells) from 24 normal individuals had geometric mean production rates of 1,779 ng for IgG, and 2,940 ng for IgM after 7 d of culture in the presence of pokeweed mitogen. By contrast, under identical conditions, lymphocytes from the 11 patients with membranous nephropathy produced significantly lower quantities of both immunoglobulins, with geometric mean concentrations of 511 ng for IgG and 439 ng for IgM. When lymphocytes from patients with membranous nephropathy were co-cultured with normal lymphocytes, the production of immunoglobulin by normal lymphocytes was depressed by 22–82%, suggesting that a population of suppressor cells was responsible for this disturbance in B-cell function. By co-culturing normal lymphocytes with patient lymphocytes depleted of either T cells or monocytes, the suppressor cell was identified as a monocyte.

INTRODUCTION

Idiopathic membranous glomerulonephropathy is a form of nephritis distinguished by the following characteristics: clinically, by an indolent course with gradual deterioration of renal function (1); pathologically, by the presence of subepithelial electron-dense deposits that, from animal studies, represent putative immune complexes formed in large antigen excess or reciprocally antibody deficiency (2); and immunopathologically, by the presence of granular deposits of immunoglobulin and complement in the glomerulus (3), giving further corroboration to the notion that this disorder arises from the entrapment of circulating immune complexes in the kidney. Of importance is the fact that current assays have not been able to demonstrate the presence of complexes in the sera of a large number of such patients (4, 5), suggesting that this may be a disease characterized by low levels of complexes. This is in contrast to other types of glomerular disorders with either mesangial proliferation of fibrin deposition (6, 7). (Alternatively, the lesions of membranous nephropathy may result from other immunopathogenetic mechanisms, such as *in situ* immune complex formation [8–11], as has been demonstrated for some models of nephritis.) Studies that have identified the presence of such complexes in the sera of a subpopulation of patients with this form of the disease, namely those with a concurrent renal vein thrombosis (12), and in the sera of occasional patients with this disorder (13), attest to the potential role of circulating immune complexes in the genesis of the renal lesion.

We propose a hypothesis that accounts for the distinctive abnormalities of this disease—that whatever the inciting etiologic agent, there is a defect in the ability of the host to mount an effective antibody response, leading to complexes being formed in large antigen excess, resulting in small-sized complexes that have been found in the serum of animals developing membranous nephropathy in the model of chronic serum-sickness nephritis (2). Further, because the available amount of antibody would be small, the concentrations of complexes generated would be correspondingly re-

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duced. The postulate that patients with membranous nephropathy have a defective antibody response also provides a rationale for the alternative mechanism of renal injury, namely, *in situ* immune complex formation; the reduced antibody concentrations would facilitate antigen implantation in the glomerulus, allowing local formation of antigen-antibody complexes. To test the validity of this hypothesis, we have examined the ability of peripheral blood lymphocytes of these patients to produce immunoglobulin in response to a polyclonal B-cell activator, pokeweed mitogen (PWM).[1] We report here the finding that lymphocytes from patients with membranous nephropathy have a diminished capacity to produce immunoglobulin when cultured in the presence of PWM. This defect is apparently the result of the presence of a population of suppressor cells that can inhibit the production of immunoglobulin by the normal cells and whose activity can be demonstrated by co-culture with normal lymphocytes. This suppressor cell has been identified as a monocyte.

[1] *Abbreviation used in this paper*: PWM, pokeweed mitogen.

METHODS

Patient selection. 11 patients, ages 17–62 yr, with a renal histological diagnosis of membranous glomerulonephropathy as defined by standard criteria, were studied (14). The biopsies showed diffuse membranous thickening with spikes after staining with the silver methanamine reagent, granular deposits of immunoglobulin, complement (C3) in only glomerular capillary loops by immunofluorescence, and subepithelial electron-dense deposits on electronmicroscopy. During the period of study, none of the patients had clinical or serologic evidence of syphilis, hepatitis, systemic lupus erythematosus, or cancer. None of the patients had received any immunosuppressive drugs (including steroids); the only medication being taken was furosemide. Their clinical and

TABLE I

Clinical and Laboratory Data of Patients with Membranous Nephropathy and of Nephrotic Controls

Patient	Age	Sex	Serum creatinine	Serum albumin	24 h protein excretion	Serum immunoglobulins		PWM-stimulated immunoglobulin produced in culture by PBL*	
						IgG	IgM	IgG	IgM
	yr		*mg/100 ml*	*g/100 ml*	*g/24 h*	*mg/100 ml*		*ng/2 × 10⁶ PBL*	
Membranous nephropathy									
1	17	F	8.0	2.9	ND‡	580	45	710	684
2	57	F	7.8	3.0	ND	625	47	778	220
3	40	M	1.4	2.6	10.3	583	110	389	541
4	62	F	1.3	2.9	4.5	492	92	745	396
5	61	F	2.1	3.1	5.9	465	55	583	242
6	62	M	2.0	3.0	3.2	427	87	601	541
7	46	M	1.0	2.2	7.4	423	71	256	655
8	40	F	1.2	2.8	5.0	362	50	333	702
9	56	F	0.8	3.2	2.4	456	87	488	286
10	41	M	1.3	2.5	ND	380	57	674	770
11	42	M	1.2	2.8	6.5	197	152	398	198
Nephrotic controls									
12	56	M	2.1	2.4	4.8	560	73	1,463	1,975
13	32	M	0.8	1.8	7.4	101	332	2,027	2,407
14	35	M	2.5	3.0	4.6	677	85	1,398	1,862
15	55	F	3.2	2.8	5.1	450	90	1,897	4,504
16	55	M	3.9	2.9	3.5	560	130	1,385	1,907
17	16	M	0.9	1.8	15.0	452	177	1,876	3,507
18	53	M	4.8	3.0	4.8	356	100	922	1,590
19	35	M	2.1	3.1	4.1	404	280	2,846	4,031
20	18	M	0.8	2.1	8.6	281	165	804	1,612
21	63	F	3.8	3.2	3.6	685	110	901	1,560

Normal range for serum immunoglobulins (geometric mean±2 SD) = IgG 560–1,509 mg/100 ml, IgM 49–261 mg/100 ml. Renal histological diagnosis for nephrotic controls: diabetic nephropathy, patients 12, 14, 15, 16, 19, 21; lipoid nephrosis, patients 17, 20; focal glomerulosclerosis, patient 18; diffuse proliferative glomerulonephritis, patient 13.

* PBL, peripheral blood mononuclear cells.

‡ ND, not done.

laboratory characteristics are detailed in Table I. 24 healthy subjects matched for sex and age were used as controls. Additionally, two disease control groups were studied: 10 patients with the nephrotic syndrome (nephrotic controls) as a result of a variety of glomerular diseases, and another 10 patients with varying degrees of chronic renal failure, as uremic controls. Their clinical and laboratory features are summarized in Tables I and II. The mean ages of the three groups were: membranous nephropathy 47.6 yr, nephrotic controls 41.8 yr, uremic controls 56.5 yr. There were no significant differences between the mean ages of the patients with membranous nephropathy compared with each of the other two groups. Four patients with membranous nephropathy were evaluated on two occasions other than the initial evaluation.

Assay system for immunoglobulin (Ig)G and IgM produced by lymphocytes in culture. This is the method described by Waldman et al. (15). Lymphocytes were isolated from fresh heparinized blood (20 U preservative-free heparin/ml of blood) by Ficoll-Hypaque density gradients (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) by the method of Boyum (16). Preparations were 90–95% viable as assessed by trypan blue dye exclusion. The lymphocytes were washed exhaustively with balanced salt solution containing 5% heat-inactivated fetal calf serum. The last wash was saved and found to be free of measurable immunoglobulin. The cells, at a concentration of 2×10^6 lymphocytes/ml in RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 2 mM glutamine, 10% decomplemented fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, were incubated in the presence of 0, 5, 10, and 20 μl PWM at 37°C in a 5% CO_2-humidified atmosphere in 13 × 100-mm plastic culture tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). A maximum response was obtained with 10 μl of PWM and results are expressed using this concentration of PWM. At the end of 7 d, culture tubes were centrifuged at 2,500 rpm for 10 min. The amount of IgG or IgM secreted into the culture media was determined by a double-antibody radioimmunoassay previously described (17). The antisera used did not show cross-reactivity as assessed by immunoelectrophoresis and immunoprecipitation with labeled antigen, precipitation occurring only with the respective antigen-specific antiserum. 1-mg aliquots of IgG or IgM were labeled with 2–3 mCi ^{125}I using a chloramine-T procedure. The dilution of rabbit anti-IgG or anti-IgM that would bind 60% of an appropriate amount of labeled antigen was found. Antigen-antibody titration curves were constructed over an antigen range of 100–1,000 ng of immunoglobulin and 1:10–1:1,000 dilution of antisera. Twice the equivalent amount of goat anti-rabbit gamma

TABLE II

Clinical and Laboratory Data of Patients with Chronic Renal Failure

Patient	Diagnosis	Age	Sex	Serum creatinine	Serum albumin	PWM stimulated immunoglobulin produced in culture by PBL*	
						IgG	IgM
		yr		*mg/100 ml*	*g/100 ml*	*ng/2 × 10^6 PBL*	
1	Diabetic nephropathy	42	M	11.1	2.8	2,797	4,796
2	Hypertensive nephrosclerosis	64	M	9.6	6.1	1,874	3,351
3	Polycystic kidneys	63	M	10.2	4.2	1,896	4,040
4	Hypertensive nephrosclerosis	62	M	8.9	3.9	855	1,017
5	Diabetic nephropathy	56	F	7.1	3.8	1,884	2,371
6	Diabetic nephropathy	68	M	10.0	3.6	1,972	3,032
7	Hypertensive nephrosclerosis	62	F	6.5	3.6	1,821	1,832
8	Polycystic kidneys	46	F	13.2	4.2	1,107	1,613
9	Polycystic kidneys	40	F	4.0	4.6	1,361	3,510
10	Diabetic nephropathy	62	F	7.6	3.1	1,587	2,235

* PBL, peripheral blood mononuclear cells.

globulin was used to precipitate the rabbit immunoglobulin. In the actual procedure, 0.05 ml of culture supernate (or standard) and 10 ng of labeled antigen were added to an equal volume of anti-IgG or anti-IgM at room temperature for 3 h. Then 0.05 ml of goat anti-rabbit gamma globulin was added with further incubation for 15 h at 4°C when 1.0 ml of 0.01 M Tris buffer pH 7.4 was pipetted to each tube. Tubes were centrifuged at 2,000 *g* for 20 min at 4°C with recovery of the pellets for counting. The binding of labeled immunoglobulin could be inhibited up to 90% by cold-specific immunoglobulin. When 90–95% of the added amount was detected, recovery experiments were also performed using known amounts of immunoglobulin.

Additionally, supernates of cell cultures from normal subjects and from patients with membranous nephropathy were assayed for immunoglobulin concentration by a solid phase fluorescence immunoassay (Immuno-Fluor, Bio-Rad Laboratories, Richmond, Calif.) previously described in detail (18). The reagents consist of derivatized polyacrylamide beads covalently coupled to rabbit anti-human heavy-chain specific antibody and a soluble fluorescein-conjugated monospecific anti-human heavy-chain antiserum. 10-μl aliquots of culture supernate (or standard supplied by the manufacturer) were added to 12 × 75-mm borosilicate glass tubes, followed by 1.0 ml of the reconstituted solid-state immunoabsorbent (Immunobead, Bio-Rad Laboratories) with mixing. The tubes were incubated at 37°C for 1.5 h. A 50-μl aliquot of the appropriate antisera was then added to each tube with further mixing. The sample was incubated at 37°C for another hour. The tubes were centrifuged at 1,700 *g* for 8 min; the supernate was decanted and the precipitate washed with 3 ml phosphate-buffered saline. The tubes were again centrifuged, supernates removed, and the precipitate resuspended in 3.0 ml phosphate-buffered saline; fluorescence in each sample was determined in a spectrofluorometer (Aminco-Bowman American Instrument Co., Silver Spring, Md.) using filters at excitation wavelength of 485 nm and emission wavelength of 525 nm. Corrected relative fluorescence equals the value of the sample minus the reading of the reagent blank. The amount of bound fluorescent antibody is directly proportional to the amount of antigen absorbed by the solid-state reagent. The immunoglobulin concentration of the sample was determined from the standard curve. Values obtained by this assay and the radioimmunoassay gave highly significant correlations: IgM, $r = 0.98$, $P < 0.01$; IgG, $r = 0.97$, $P < 0.01$.

For co-culture experiments, the techniques described by Broder et al. (19) were used. Using identical conditions as described above, an equal number (1×10^6) of lymphocytes from both normals and patients with membranous nephropathy were co-cultured in the presence of 10 μl of PWM. The results were expressed as percent of expected immunoglobulin (Ig) produced by cells in co-culture:

$$\frac{\text{Amount of Ig produced by cells in co-culture}}{1/2 \times (\text{sum of Ig produced by cells of each individual culture separately})} \times 100.$$

Percent suppression of immunoglobulin produced = 100 − percent of expected immunoglobulin produced by cells in co-culture. 10 experiments were done using lymphocytes as controls from paired normal subjects. Co-cultures were also done using lymphocytes from uremic and nephrotic control patients mixed with normal lymphocytes. Cultures were also performed using 2×10^6 lymphocytes from each subject (4×10^6 cells in 2 ml of medium) with similar results. All experiments were done in duplicate, incorporating control and patient samples in each run.

Evaluation of the suppressor cell. To determine whether the suppressor cell was a mononuclear phagocyte cell or a T cell, or both, cultures and co-cultures were done concurrently, using lymphocytes depleted selectively of each cell type. To remove phagocytes, lymphocytes were prepared by prior incubation of 50 ml of blood with 250 mg sterile carbonyl iron powder (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, N. Y.) at 37°C for 30 min in 5% CO_2 with continuous agitation. Iron-ingested cells were removed by differential centrifugation using Ficoll-Hypaque gradients. The resultant lymphocyte preparations contained <1% monocytes, as assessed by latex particle ingestion and morphology (20).

Lymphocytes were depleted of T cells by removing sheep erythrocyte receptor-bearing lymphocytes in Ficoll-Hypaque density gradients (17). Plastic tubes containing 0.5 ml of 5×10^6 lymphocytes/ml suspension were incubated with an equal volume of 0.5% sheep erythrocytes for 5 min at 37°C. Mixtures were centrifuged at 200 *g* for 5 min, followed by incubation for 4 h at 4°C. The erythrocyte-lymphocyte pellets were dispersed, pooled, and layered onto a Ficoll-Hypaque mixture (three parts 9% Ficoll and two parts 35% Hypaque). Centrifugation was done at 400 *g* for 40 min, and cells removed from the interface. The procedure was repeated with these cells and a B-cell-enriched fraction was obtained containing about 1–3% erythrocyte rosette-forming cells as determined by re-rosetting with sheep erythrocytes. Later experiments were done using the procedure described by Saxon et al. (21), pretreating sheep erythrocytes with 2-aminoethyl-isothiouronium bromide hydrobromide.

Quantitation of peripheral blood mononuclear subpopulations. T cells were identified by their ability to form rosettes with sheep erythrocytes (22), B cells by their ability to form rosettes with sheep erythrocytes sensitized with IgM antibody and mouse (C3) number 3 (23). Monocytes were identified by morphology and by latex particle ingestion. These determinations were done in seven patients.

Measurement of serum immunoglobulins. This was done by radial immunodiffusion (24) using commercial antisera (Calbiochem-Behring Corp., La Jolla, Calif.) and stabilized human serum (Calbiochem-Behring Corp.) as a standard. All experiments were done in duplicate.

Statistical analysis. Statistical analysis was performed by *t* test.

RESULTS

Serum immunoglobulin levels. The geometric means for serum immunoglobulin levels in patients with membranous nephropathy were 436 mg/100 ml for IgG and 72 mg/100 ml for IgM. The levels were significantly lower when compared with the geometric means of normal subjects: 919 mg/100 ml for IgG ($t = 6.8$, $P < 0.01$), 114 mg/100 ml for IgM ($t = 3.0$, $P < 0.01$; Table I). For nephrotic control patients (Table I) the geometric means were 406 mg/100 ml for IgG and 136 mg/100 ml for IgM, the former value being significantly lower than the normal geometric mean ($t = 4.4$, $P < 0.01$).

Enumeration of peripheral blood mononuclear cell subpopulations. The values (mean±SE) for seven patients were 75.6±1.6% for T cells, 17.4±2.6% for B cells, and 10.3±0.7% for monocytes. These were within the normal ranges observed: T cells 63–78%, B cells 5–21%, monocytes 6–17%.

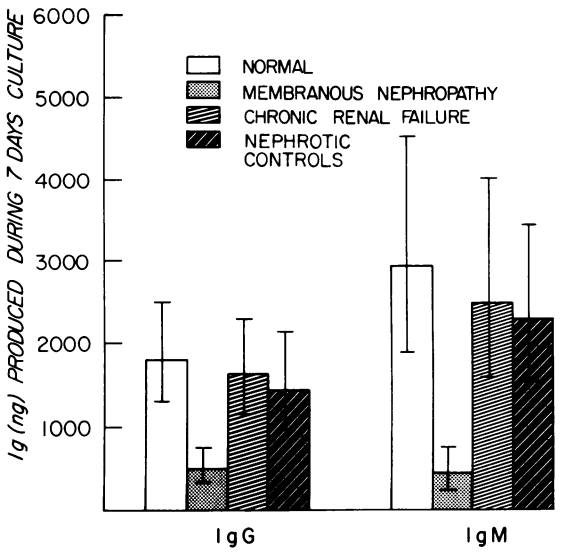

FIGURE 1 Quantities of IgG and IgM produced by 2×10^6 peripheral blood lymphocytes after 7 d culture in the presence of 10 μl PWM. Four groups of subjects were studied: 24 healthy controls, 11 patients with membranous nephropathy, 10 patients with chronic renal failure, and a similar number with miscellaneous forms of renal disease. The group with membranous nephropathy produced significantly lower amounts of IgG and IgM compared with the control group. Results show the geometric mean concentrations in nanograms ±1 SD.

In vitro synthesis of IgG and IgM by peripheral blood lymphocytes Fig. 1. The geometric means of immunoglobulin production of normal subjects were 1,779 ng for IgG and 2,940 ng for IgM (per 2×10^6 cells after 7 d culture in the presence of 10 μl PWM). The peripheral blood lymphocytes of patients with membranous nephropathy produced significantly lower amounts of both immunoglobulins, having geometric means of 511 ng for IgG ($t = 9.7$, $P < 0.01$) and 439 ng for IgM ($t = 10.3$, $P < 0.01$). This defect in immunoglobulin production was persistent in the four subjects tested on two consecutive occasions at monthly intervals.

Lymphocytes from uremic patients and nephrotic control patients produced geometric mean concentrations of 1,636 and 1,442 ng IgG, respectively; the corresponding values for IgM for each group were 2,536 and 2,308 ng. These were not significantly different compared with normal values. The individual values of the patients are given in Tables I and II.

Co-culture experiments. To determine whether circulating suppressor cells were responsible for this defect in immunoglobulin production, co-culture experiments were done. The results are shown in Table III. In experiments done with mixtures of lymphocytes from the patients with membranous nephropathy and normal subjects, percent suppression ranging from 41 to 81% was observed for lymphocytes from 10 patients with regard to IgG production; lymphocytes from all 11 patients demonstrated significant suppressing capacity for IgM, ranging from 22 to 82%. In 10 co-culture experiments with lymphocytes from normal subjects, the percent suppression did not exceed 10% for IgG and 7% for IgM. The results of co-culture experiments between normal lymphocytes and cells

TABLE III
Suppression of Immunoglobulin Production of Normal Peripheral Blood Lymphocytes by Lymphocytes from Patients with Membranous Nephropathy

Experiment	IgG ng/2 × 10^6 lymphocytes				IgM ng/2 × 10^6 lymphocytes			
	Normal	Patient	Patient + normal	Inhibition	Normal	Patient	Patient + normal	Inhibition
				%				%
1	1,898	710	722	45	1,260	684	756	22
2	2,054	778	445	69	1,365	220	176	78
3	1,855	389	599	47	3,520	541	486	76
4	855	745	252	41	1,968	396	573	52
5	2,797	583	1,562	8	1,980	242	435	60
6	1,021	601	426	48	1,760	541	512	55
7	2,054	256	223	81	2,874	655	527	70
8	2,254	333	522	60	2,068	702	254	82
9	1,454	488	556	43	3,438	286	507	73
10	3,175	674	604	69	4,466	770	1,716	34
11	2,132	398	653	48	3,164	198	624	63

Normal lymphocytes (1×10^6 cells) were mixed with an equal number of lymphocytes from 11 patients with membranous nephropathy and cultured for 7 d in the presence of 10 μl of PWM. The percent inhibition of expected immunoglobulin production was calculated as described in Methods. 10 control co-cultures were done using lymphocytes from pairs of normal subjects. Percent inhibition did not exceed 10% for IgG and 7% for IgM in control experiments.

from nephrotic control patients are shown in Table IV. Suppressive activity did not exceed 8% for IgG and 7% for IgM. Lymphocytes from five uremic subjects tested did not exhibit significant suppressive activity: 9% for IgG and 8% for IgM.

Experiments using fractionated lymphocytes to determine the identity of the suppressor cell. The results are shown in Table V. When normal lymphocytes were co-cultured with patient lymphocytes subtracted of T cells, suppression was maintained at the same level as with unfractionated lymphocytes. However, when monocytes were removed, the suppressive activity was completely abolished. Additionally, in two experiments, when patient lymphocytes (2×10^6 cells) were depleted of monocytes and cultured alone, the production of immunoglobulin rose, in one case, from 410 to 1,412 ng IgG and, in the other case, from 387 to 1,760 ng IgG. In comparison, the results of culture and co-culture experiments using lymphocytes from nephrotic control patients are shown in Table VI. No significant increase in immunoglobulin production resulted from the removal of monocytes in these experiments.

DISCUSSION

The present study clearly delineates a defect in the ability of peripheral blood lymphocytes from patients with membranous nephropathy to produce normal amounts of IgG and IgM on stimulation with PWM. The defect was not related to either the renal functional status of the patient or the nephrotic state per se because control experiments done with lymphocytes from patients who were either uremic or nephrotic did not reveal similar findings. Additionally, investigations by others have shown normal immunoglobulin synthesis by lymphocytes from patients with lipoid nephrosis (25). The inability of peripheral blood lymphocytes from patients with membranous nephropathy to produce adequate amounts of immunoglobulin was shown to be the result of a population of suppressor cells whose activity could be demonstrated, when normal immunoglobulin production was suppressed, by co-culturing with normal lymphocytes. By mixing experiments using lymphocytes depleted selectively of either T cells or monocytes, identification of the monocyte as the suppressor cell was established. Suppressor activity was nullified only with removal of monocytes. From studies of patients with multiple myeloma and sarcoidosis (17, 26), there is precedent for the monocyte as a suppressor cell. The monocyte has been found to elaborate a variety of immunoregulatory substances, some of which inhibit lymphocyte function (27).

The significance of these observations relates to the partial validation of the hypothesis proposed. The restrictions on the interpretation of the data are acknowledged. The function of only one pool of lymphocytes is being tested. Although PWM has been used previously to explore the cellular aberrations of patients with a variety of immune disorders (15, 17, 19, 26, 28–32), there is only limited information to indicate that a defective B-cell response to this substance is paralleled by a diminished response of these cells to antigenic challenge (17). It is also apparent that the demonstration of a poor antibody response to antigen-

TABLE IV

Results of Co-Culture Experiments between Lymphocytes from Normal Subjects and Nephrotic Control Patients

Experiment	IgG ng/2 × 10^6 lymphocytes				IgM ng/2 × 10^6 lymphocytes			
	Normal	Patient	Patient + normal	Inhibition	Normal	Patient	Patient + normal	Inhibition
				%				%
1	2,132	1,463	1,840	−2	3,164	1,975	2,781	−10
2	1,998	2,027	1,901	6	3,892	2,407	2,973	6
3	2,054	1,398	1,856	−8	3,322	1,862	2,436	6
4	1,454	1,897	1,862	−11	1,980	4,504	3,092	−5
5	1,687	1,385	1,384	−10	2,420	1,907	2,346	−9
6	1,504	1,876	1,819	−8	2,874	3,507	3,040	5
7	2,087	922	1,384	8	4,092	1,590	2,654	7
8	1,974	2,846	2,650	−10	2,940	4,031	3,821	10
9	1,980	804	1,451	−5	3,366	1,612	2,815	6
10	2,797	901	1,751	6	5,412	1,560	3,712	−7

For 7 d, in the presence of 10 μl PWM, lymphocytes (1×10^6 cells) from normal persons were cultured with an equal number of cells from patients with various forms of renal disease. Percent inhibition was calculated as described in Methods. Negative percentages denote that the amount of immunoglobulin produced exceeded the amount expected.

TABLE V

Effect of Cell Subpopulations from Patients with Membranous Nephropathy on Suppression of Immunoglobulin Production by PWM-stimulated Lymphocytes of Normal Subjects

Responder cells	Source of suppressor cell	Suppressor cell fraction depleted of	PWM-stimulated Ig produced in culture by PBL*		
			IgG	IgM	Suppression
			ng/2 × 10⁶ cells		%
PBLN1				1,584	
	MGN patient 2‡			183	
PBLN1	MGN patient 2	Unfractionated		272	69
PBLN1	MGN patient 2	E-RFL depleted§		216	75
PBLN1	MGN patient 2	Monocyte depleted		1,812	−105
PBLN2			2,242		
	MGN patient 3		246		
PBLN2	MGN patient 3	Unfractionated	460		63
PBLN2	MGN patient 3	E-RFL depleted	405		67
PBLN2	MGN patient 3	Monocyte depleted	2,455		−94
PBLN3				3,514	
	MGN patient 7			420	
PBLN3	MGN patient 7	Unfractionated		340	82
PBLN3	MGN patient 7	E-RFL depleted		410	79
PBLN3	MGN patient 7	Monocyte depleted		2,816	−44
PBLN4			1,762		
	MGN patient 9		289		
PBLN4	MGN patient 9	Unfractionated	410		60
PBLN4	MGN patient 9	E-RFL depleted	503		51
PBLN4	MGN patient 9	Monocyte depleted	1,611		−57

For 7 d, in the presence of 10 μl PWM, lymphocytes (1 × 10^6 cells) from normal persons were cultured with an equal number of cells from patients with membranous nephropathy. Percent inhibition was calculated as described in Methods. Negative percentages denote that the amount of immunoglobulin produced exceeded the amount expected.

* PBL, peripheral blood mononuclear cells.

‡ MGN, membranous glomerulonephropathy.

§ E-RFL, erythrocyte rosette-forming lymphocytes.

specific challenge would be the critical observation. However, the nature of the antigen(s) in this form of progressive glomerulonephritis is unknown, and the relative unresponsiveness of lymphocytes to a polyclonal B-cell stimulation with PWM provides reasonable evidence of a B-cell defect in these patients. These findings are consistent with the pathogenetic mechanisms responsible for the production of membranous nephropathy in one of the animal models studied, namely, chronic serum-sickness nephritis.

In chronic serum-sickness nephritis, the classic studies of Dixon et al. (33) clearly demonstrate that only rabbits exhibiting a poor antibody response to the inciting antigen would develop progressive renal disease. These observations were extended by Germuth and Rodriguez (2) who related the morphologic expression of nephritis to the size of the circulating immune complex and showed that membranous nephropathy occurred in animals developing a very poor antibody response, allowing the production of small-sized immune complexes formed presumably in large antigen excess. The results of another study provide additional support for Germuth's hypothesis. In an investigation using the active serum-sickness nephritis model, the serum of the single animal developing membranous nephropathy was shown to contain an immune complex of relatively small size (34). Parenthetically, it must be stated that the size of the antibody response exerts a critical role only when constant doses of antigen are given to the animal (35). The ratio of antigen to antibody is the more critical determinant (35). Other properties of either the antigen (such as its valence) or the antibody (affinity for the antigen) have also been shown to be important in determining the localization of complexes in the glomerulus, probably by influencing their size (36). Clinically, patients with lupus membranous nephropathy have been shown to be associated with nonprecipitating

TABLE VI

Effect of Monocyte Depletion on PWM-stimulated Immunoglobulin Production by Lymphocytes from Nephrotic Control Patients during Culture and Co-Culture with Normal Lymphocytes

Experiment	Patient cells	Normal cells	PWM-stimulated Ig produced in culture by PBL* IgG	IgM	Suppression
			ng/2 × 10⁶ cell		%
1	Patient 2		2,250		
2	Patient 2—monocyte depleted		2,025		11
3		Normal 1	2,033		
4	Patient 2	Normal 1	2,256		6
5	Patient 2—monocyte depleted	Normal 1	2,362		4
1	Patient 4		1,726		
2	Patient 4—monocyte depleted		1,850		−7
3		Normal 2	1,613		
4	Patient 4	Normal 2	1,589		5
5	Patient 4—monocyte depleted	Normal 2	1,690		−6
1	Patient 1			2,016	
2	Patient 1—monocyte depleted			1,844	9
3		Normal 3		2,965	
4	Patient 1	Normal 3		2,681	−8
5	Patient 1—monocyte depleted	Normal 3		2,824	−5
1	Patient 8			3,985	
2	Patient 8—monocyte depleted			3,689	10
3		Normal 4		2,817	
4	Patient 8	Normal 4		3,201	6
5	Patient 8—monocyte depleted	Normal 4		3,078	4

* Peripheral blood mononuclear (PBL) cells (2×10^6 cells) were cultured either by themselves or after monocyte depletion. Unfractionated or fractionated patient cells (1×10^6 cells) were co-cultured with an equal number of normal cells; all cultures were done with 10 μl PWM for 7 d. Percent inhibition was calculated as in Methods. Negative percentages denote that the amount of immunoglobulin produced exceeded that expected.

anti-DNA antibodies in their sera (37, 38). The study also showed that patients with lupus membranous nephropathy had significantly lower titers of serum anti-DNA antibody compared with patients with other forms of lupus nephritis, an observation that supports the current findings.

Mention must be made of the alternative model of membranous nephropathy, namely, Heyman's nephritis. Recent experiments have demonstrated that renal injury in this model results from the interaction of antibody with an antigen that is an integral component of glomerular structure (10, 11). The relevance of our observations to this pathogenetic mechanism of membranous nephropathy is uncertain; at best they may provide an explanation for the indolent course of the illness.

Finally, it is acknowledged that the results of the present study do not exclude the presence of other potential mechanisms that may interfere with the immune responses, such as the presence of serum factors having immunoregulatory functions.

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