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Immunodynamics of minimal change nephrotic syndrome in adults T and B lymphocyte subsets and serum immunoglobulin levels

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SUMMARY

Thirty-two adult patients with minimal change nephrotic syndrome (MCNS) were studied in order to clarify the characteristics of the immune system in MCNS and their relation to clinical activity. In the active phase ($n=17$), serum immunoglobulin (Ig) M and E levels, B lymphocytes (surface Ig-positive cells) and their subsets, surface IgG, IgM and IgE positive cells; Bγ, Bμ and Bε, were increased, whereas the serum IgG level and OKT3-reactive cells, peripheral T lymphocytes; T3, were decreased. In the remitted phase maintained by steroid therapy ($n=17$), serum Igs and B lymphocyte subsets tended to return to normal levels concomitant with decreases in T3 and T4, and an increase in T8 in consequence of a marked decrease in T4/T8 (helper/suppressor) ratio. In stable remission continuing with no steroid therapy ($n=14$), the above abnormalities returned to normal ranges, except for serum IgM which remained at a high level and re-elevated serum IgE. These results suggest that immunological abnormalities in MCNS are characterized by acceleration of the IgE and IgM producing systems and impaired maturation of the IgG producing system despite normal differentiation from the IgM producing to IgG producing system, possibly caused by T lymphocyte dysfunction.

Keywords T and B lymphocyte subpopulations and subsets immunoglobulins corticosteroid minimal change nephrotic syndrome

INTRODUCTION

Associations of minimal change nephrotic syndrome (MCNS) with pollen allergy (Hardwicke *et al.*, 1959; Wittig & Goldman, 1970) and increased serum immunoglobulin (Ig) E level were reported as evidences of dysfunction of the humoral immune system in this disease (Groshung *et al.*, 1973). On the other hand, from the viewpoint of cellular immunity, it was suggested that abnormality of T lymphocyte function might be involved in the pathogenesis of MCNS as the priniciple abnormality (Shalhoub, 1974). In addition, a suppressed responsiveness to mitogens of patient's lymphocytes, or normal donor lymphocytes coexisting with the patient's sera was reported (Moorthy *et al.*, 1976; Tomizawa *et al.*, 1979; Sasdelli *et al.*, 1980). However, the causal relationship between such abnormalities of humoral and cellular immunity in MCNS remains as yet largely unknown.

In our previous study (Tani *et al.*, 1982), we reported that various primary glomerulonephritides disclosed individual characteristic response patterns of B lymphocytes, which are assumed to play an important role in determining each glomerular histological type in idiopathic glomerulonephri-

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tides. In the current study, we focused on MCNS and have correlated the changes of peripheral T and B lymphocyte subsets and serum Ig levels with disease activity.

MATERIALS AND METHODS

Patients

Thirty-two patients with histologically confirmed MCNS (19 males and 13 females, range and mean of ages; 15–63, 31·5 years), and 24 healthy individuals (23 males and one female; 15–35, 27·2 years) serving as the control group, were included in this study. The former were staged into three phases according to disease activity at the time they were first studied.

Nephrotic phase, (17 cases). Condition with proteinuria more than 3·5 g/day and with serum albumin less than 30 g/l. The group was further divided into two subgroups.

(a) Initial episode, (nine cases). Initial episodes of MCNS and its recurrences after stable remission with no necessity of steroid therapy.
(b) Relapse, (eight cases). Recurrences during steroid therapy.

Unstable remission, (17 cases). Disappearance of urinary protein and normalization of serum albumin, requiring steroid therapy for maintenance remission.

Stable remission, (14 cases). Remission maintained despite withdrawal of steroid.

Therapy

The patients were treated with prednisolone 40 mg daily for 4 weeks, followed by gradual tapering to a maintenance dose of 10 mg daily according to the clinical activity.

Immunological study

Lymphocyte subpopulations and subsets. Mononuclear cells were separated from heparinised peripheral venous blood by the Ficoll-Hypaque gradient centrifugation method (400 $g \times 30$ min) and adjusted to 1×10^7 cells/ml in phosphate-buffered saline (PBS: pH 7·4).

(a) T lymphocyte subpopulation and subsets. These were identified by rosette formation by rosette formation with sheep erythrocyte (E-RFC), and by the indirect immunofluorescence technique using monoclonal antibodies OKT3 (peripheral T; T3), OKT4 (helper/inducer; T4) and OKT8 (suppressor/cytotoxic; T8, Ortho). The latter was performed as follows; 50 μl of the cell suspension was mixed with 5 μl of each monoclonal antibody and incubated for 30 min in an ice water bath followed by washing with PBS. An appropriately diluted fluorescein isothiocyanate (FITC)-labelled anti-mouse IgG goat IgG/F(ab′) 2 solution was then added and incubated for a further 30 min. The cells were washed, resuspended in 30% glycerol and subjected to fluoroscopic examination (BHB-RFL, Olympus). The percentage of positive cells was recorded after counting more than 200 cells.
(b) B lymphocyte subpopulation and subsets. Surface immunoglobulin (sIg) positive cells identified by the direct immunofluorescence technique were regarded as B cells. Extrinsic Igs adhering to cell surface receptors were detached by a treatment with acetate buffered saline pH 4·0 for 1 min (Kumagai *et al.*, 1975) prior to 30 min incubation in an ice water bath with appropriately diluted FITC-labelled anti-human Ig (IgG + IgA + IgM), IgG (γ-chain), IgA (α-chain), IgM(μ-chain) rabbit IgG/F(ab′)2 antibodies and IgE (ε-chain) rabbit antiserum (Behringwerke). Positive cells were defined as B, Bγ, Bα, Bμ or Bε, and the percentage of each recorded after counting more than 400 cells.

Serum immunoglobulin levels. Serum IgG, IgA and IgM levels were determined using laser nephelometry (Behringwerke), and serum IgE levels by the radioimmunosorbent test (Pharmacia). All immunological studies in the nephrotic phase were performed before initiating steroid therapy. Statistical significances were determined by Student's *t*-test.

RESULTS

T lymphocyte subpopulation and subsets. In the nephrotic phase, E-RFC and T3 decreased significantly ($P < 0{\cdot}05$), especially in relapsed (Table 1). However, there was no apparent change in

Table 1. T lymphocyte subsets in minimal change nephrotic syndrome

	No. of patients	E-RFC (%)	T3 (%)	T4 (%)	T8 (%)	T4/T8 ratio
Nephrotic syndrome	14	58·3±1·7*	53·6±2·0*	35·3±1·7	23·0±1·5	1·60±0·11
Initial episode	7	60·2±3·6	56·0±2·2	33·8±2·3	21·3±2·2	1·67±0·17
Relapse	7	57·4±2·0*	51·3±3·3*	36·7±2·1	24·7±1·8	1·53±0·07
Unstable remission	17	59·6±1·7	51·5±2·2†	26·8±1·9‡	27·0±1·1†	1·01±0·07‡
Stable remission	11	63·6±3·5	56·6±1·3	35·0±1·3	22·2±1·3	1·64±0·11
Control	17	63·7±2·4	60·3±2·3	39·1±2·1	22·2±1·2	1·74±0·13

* $P<0·05$, † $P<0·01$, ‡ $P<0·001$. (Mean±s.e.)

T4, T8 and T4/T8 ratio. In unstable remission, E-RFC tended to decrease. T3 and T4 were significantly decreased ($P<0·01$, respectively), whereas T8 was increased significantly ($P<0·01$), resulting in a marked decrease in T4/T8 ratio ($P<0·01$). However, these were normalized when stable remission was achieved.

B-lymphocyte subpopulation and subsets. In the nephrotic phase, B, Bγ, Bμ and Bε were significantly increased (Table 2). Especially in the initial episode, increases in Bμ and Bε were marked ($P<0·02$, $P<0·005$, respectively). However, these tended to return to normal levels in unstable remission, and normalized in stable remission.

Table 2. B lymphocyte subsets in minimal change nephrotic syndrome

	No of patients	B cell (%)	Bγ (%)	Bα (%)	Bμ (%)	Bε (%)
Nephrotic syndrome	17	21·2±1·9†	12·1±1·2‡	6·0±0·7	5·5±0·5*	3·11±0·65‡
Initial episode	9	21·8±1·8‡	12·1±1·4‡	5·8±0·8	5·9±0·7†	3·76±1·90§
Relapse	8	20·6±2·4*	12·0±2·0*	6·2±1·1	5·0±0·6	2·39±0·90
Unstable remission	17	20·9±1·4‡	11·5±1·0‡	6·9±0·5‡	5·1±0·7	1·89±0·40
Stable remission	14	16·4±1·3	8·9±0·9	4·6±0·4	4·1±0·5	1·22±0·15
Control	24	15·4±1·3	7·4±0·7	5·2±0·4	4·1±0·3	1·12±0·12

* $P<0·05$, † $P<0·02$, ‡ $P<0·01$, § $P<0·005$. (Mean±s.e.)

Table 3. Serum immunoglobulin levels in minimal change nephrotic syndrome

	No of patients	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	IgE* (IU/ml)
Nephrotic syndrome	17	642±88‡	240±33	267±28‡	2·62±0·10‡
Initial episode	9	521±110§	249±54	263±39§	2·87±0·11§
Relapse	8	891±78‡	229±36	269±41†	2·34±0·12‡
Unstable remission	17	1051±73†	218±21	234±25‡	2·36±0·07‡
Stable remission	14	1464±86	222±20	186±21a†	2·55±0·13‡
Control	17	1276±54	199±10	126±13	1·96±0·08

* Expressed in log IgE, † $P<0·02$, ‡ $P<0·005$, § $P<0·001$. (Mean±s.e.)

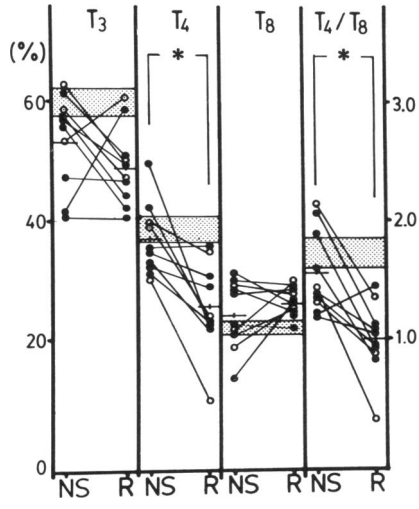

Fig. 1. Steroid induced alterations of T lymphocyte subsets in 10 patients with minimal change nephrotic syndrome during nephrotic phase (NS), initial episode (○) and relapse (●), and in remission (R). The shaded areas indicate normal ranges (mean ± s.e. of the control group), the horizontal lines denote the mean values, and the asterisks significant changes (*$P < 0.05$).

Serum immunoglobulin levels. In the nephrotic phase, IgM and IgE levels were significantly elevated ($P < 0.005$, Table 3), but the IgG level was markedly reduced ($P < 0.005$). These abnormalities were especially marked during the initial episode. The abnormalities, however, tended to normalize in unstable remission, and IgG returned to the normal range in stable remission. However, IgM still remained at a high level and IgE was re-evaluated.

Steroid-induced alterations. Immunological changes associated with administration of steroids were compared between the nephrotic phase and the phase of unstable remission induced by administration of prednisolone 40 mg daily for 4 weeks.

(a) T lymphocyte subsets. During steroid induced remission T3 tended to decrease, and T4

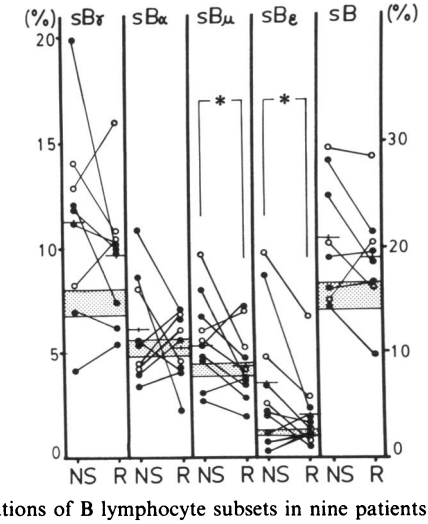

Fig. 2. Steroid induced alterations of B lymphocyte subsets in nine patients with minimal change nephrotic syndrome during nephrotic phase (NS), initial episode (○) and relapse (●), and in remission (R). The symbols are same as shown in Fig. 1. (*$P < 0.05$).

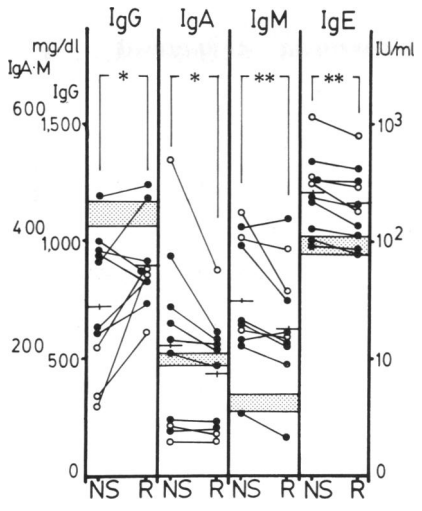

Fig. 3. Steroid induced alterations of serum immunoglobulin levels in 10 patients with minimal change nephrotic syndrome during nephrotic phase (NS), initial episode (○) and relapse (●), and in the remission (R). The symbols are same as shown in Fig. 1. (*$P<0{\cdot}05$, **$P<0{\cdot}005$).

decreased significantly ($P<0{\cdot}05$), concomitant with a marked decrease inT4/T8 (helper/suppressor) ratio from 1·56 to 1·00 ($P<0{\cdot}05$; Fig. 1).

(b) B lymphocyte subsets. Bγ and Bα tended to decrease, and Bμ and Bε decreased significantly ($P<0{\cdot}05$; Fig. 2).

(c) Immunoglobulin levels. A significant increase in IgG ($P<0{\cdot}05$) and decreases in IgA, IgM and IgE ($P<0{\cdot}05$, $P<0{\cdot}005$, $P<0{\cdot}005$, respectively) led to an overall tendency to normalization of serum immunoglobulin levels (Fig. 3).

Correlation between B lymphocyte subsets and serum Ig levels. Bγ was inversely related to serum IgG levels ($r=-0{\cdot}41$; $P<0{\cdot}005$). However, no relationship was found between the other B lymphocyte subsets and their corresponding serum immunoglobulins.

DISCUSSION

In the nephrotic phase of MCNS, increased IgM and IgE and decreased IgG levels have already been described (Giangiacomo *et al.*, 1975; Shakib *et al.*, 1977; Groshung *et al.*, 1973). For the alteration of B lymphocyte subsets, however, only a few data have been accumulated. B lymphocytes detected by Kerpen *et al.* (1979) as C3 receptor positive cells (EAC), and by Sasdelli *et al.* (1980) as EAC and sIg postive cells, were reported to have increased in the nephrotic phase. In addition, we showed in our previous paper that B lymphocytes, especially Bγ and Bε, were increased in the nephrotic phase and that the increase was the proper response pattern of the B lymphocyte subset in MCNS (Tani *et al.*, 1982).

The present results have further shown that, in the nephrotic phase, Bμ were also increased in addition to Bγ and Bε. B lymphocyte subsets are thought to be precursors of corresponded Ig producing cells (Kuritani & Cooper, 1982). Thus it is conceivable that the increase in Bμ and Bε observed in MCNS may reflect an elevation in serum IgM and IgE levels. However, in this study, Bγ and serum IgG showed an inverse correlation. As the cause of decrease in serum IgG levels, Giangiacomo *et al.* (1975) suggested, based on their data of high serum IgM and low IgG levels, a disordered differentiation from IgM producing cells to IgG producing cells, which was primarily affected by T lymphocyte dysfunction. However, our results showed increases in both Bγ and Bμ in the nephrotic phase, indicating a preserved switching mechanism from Bμ to Bγ. On the other hand, Heslan *et al.* (1982) reported decreased IgG production *in vitro* by the lymphocytes obtained from

patients in the nephrotic phase and Shakib *et al.* (1977) described a disproportionate decrease in subclasses of serum IgG, indicating that not only increased urinary excretion of Igs, which may be a widely accepted explanation, but also an impaired Ig production system are involved in the discrepancy between the increased Bγ and the low serum IgG level. It is also possible to explain the discrepancy by suggesting that Bγ is mobilized to compensate for the decrease in the serum IgG level which may be caused by a maturation defect from Bγ to IgG-producing cells as well as increased urinary loss.

It is well known that the serum IgE level is characteristically high in MCNS, especially in the nephrotic phase (Groshung *et al.*, 1973). However, the elevated IgE may not directly affect the permeability of the glomerular capillary wall, but an underlying immunological abnormality, possibly T lymphocyte dysfunction, may induce both an elevation of serum IgE and an increase in the permeability of the glomerular capillary walls, since in the current study, not all patients showed high serum IgE (Fig. 3) and tapering of or withdrawal from steroids often caused an increase in serum IgE without reappearance of clinical symptoms. In addition, several conditions accompanied by immunodeficiency syndromes, such as the Wiscott-Aldrich syndrome and thymic alymphoplasia, are known to show a high serum IgE level without excretion of urinary protein (Waldmann *et al.*, 1972; Kikkawa *et al.*, 1973).

It is well accepted that lymphocytes obtained from patients in the nephrotic phase of MCNS respond poorly to phytohaemagglutinin and concanavalin A, and that patient's sera suppress the responsiveness of normal donor lymphocytes to these mitogens (Moorthy, Zimmerman & Burkholder, 1976; Tomizawa *et al.*, 1979; Sasdelli *et al.*, 1980). Literature on the behaviour of T lymphocyte subsets in MCNS, however, is scarce. Trompeter *et al.* (1978) observed no apparent change of T lymphocyte subsets in the nephrotic phase, but an increase in Tγ (IgG-Fc receptor positive T lymphocytes) in the steroid induced remitting phase. However, Kerpen *et al.* (1979) pointed out decreased E-RFC in the nephrotic phase and its normalization in the remitted phase. Our data showing the decrease in E-RFC and T3 in the nephrotic phase corresponded with those reported by Kerpen *et al.* (1979), but no apparent change in T4 and T8 was observed during the nephrotic phase despite activation of B lymphocytes. In the phase of steroid-induced remission, a decrease in helper/suppressor ratio resulting from a decrease in T4 and increase in T8 was noted. These results suggest that the possible low suppressor function existing in the nephritic phase, which is not reflected by the T8 level, seems to be corrected by administration of steroid hormone concomitant with clinical remission.

Steroid administration, in this study corrected, or tended to correct, abnormalities not only of T lymphocyte subsets but also of B lymphocyte subsets and serum Ig levels, thereby causing improvement of clinical symptoms. Disorders of B lymphocyte function and the Ig producing mechanism, which are controlled by T lymphocytes, may be involved in the etiology of MCNS, and steroid might correct these disorders.

In summary, immunological disorders in MCNS are characterized by acceleration of the IgE and IgM producing systems and low IgG levels, possibly caused by T lymphocyte dysfunction.

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