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Immunoregulatory treatment for minimal change nephrotic syndrome

K P MEHTA, U ALI, M KUTTY, AND U KOLHATKAR

Nephrology Division and Research Laboratory, Bai Jerbai Wadia Hospital for Children, Parel, Bombay, India

SUMMARY Immunological studies were performed in 18 children with minimal change nephrotic syndrome proved by biopsy examination during relapse and in 15 age matched controls. All 18 children showed dysfunction of cell mediated immunity as evidenced by low absolute lymphocyte count, low blastogenesis index in response to phytohaemagglutinin stimulation, and reduced skin sensitivity to dinitrochlorobenzene when compared with controls. All 18 patients had low serum IgG concentrations, while the IgA, IgM, and C_3 concentrations in the serum were within normal limits.

Based on the evidence of depressed cell mediated immunity, 14 patients with nephrotic syndrome were treated with an immunoregulatory drug *l*-tetramisole (levamisole) for a period of 20–24 weeks. Six patients went into complete remission within 4–20 weeks of treatment, a further six patients went into partial remission, while two did not respond.

On follow up (six to 24 months after stopping levamisole), of the six patients who achieved complete remission, four continued to maintain the state and two relapsed after roughly six months. Of the six patients who achieved partial remission, two went into complete remission, two continued to be in partial remission, and two relapsed.

A variety of immunological alterations have been described in minimal change nephrotic syndrome.[1–3] Conventional treatment with steroids and immunosuppressants, although inducing a remission of the nephrotic state, increases the risk of severe life threatening infections in these children by further depressing the cell mediated immunity.

This study was undertaken to evaluate the immunological state of children with minimal change nephrotic syndrome in relapse who were not being given any immunosuppressive treatment and to assess the role of levamisole, an immunoregulatory drug, in the management of this disease in those who showed evidence of dysfunction in cell mediated immunity.

Patients and methods

Eighteen patients with nephrotic syndrome and 15 controls, all aged between 1 and 12 years, were studied.

All 18 patients had anasarca with proteinuria $\geq$40 mg/m^2/24 hours, hypoalbuminaemia $\leq$2·5 g/dl and hypercholesterolaemia $\geq$200 mg/dl. Histology of the renal biopsy specimen obtained percutaneously showed a minimal lesion in all 18 cases on light microscopy; the immunofluorescence was negative in all except one, which had faint deposits of IgM in mesangium.

The patients were further classified according to whether they had (a) single attacks, (b) infrequent relapses (<three a year), (c) frequent relapses (> two in six months or > three a year). None of them were receiving any immunosuppressants at the time of the study. Only two had earlier received cyclophosphamide, and that was more than two years before the present study. Immunological studies were undertaken on all these patients.

Tests for cell mediated immunity. These were (1) absolute T lymphocyte count by sheep red blood cell rosette formation; (2) blastogenesis by phytohaemagglutinin stimulation; and (3) skin testing for delayed hypersensitivity to neoantigen dinitrochlorobenzene.

Tests for humoral immunity. These were (1) absolute B lymphocyte count by rosette formation; and

(2) estimation of immunoglobulins IgG, IgM, and IgA in serum by electroimmunoassay (Rocket technique).

Complement system. Estimation of C_3 component in serum was by single radial immunodiffusion.

Treatment with levamisole. Fourteen patients were given levamisole (2·5 mg/kg body weight) as a single dose at bed time either daily or on alternate days for a period of 20–24 weeks. During this period they were observed clinically with special reference to weight, blood pressure, oedema, and the number and severity of infections. Laboratory monitoring was performed weekly by blood count and complete urinalysis and monthly for blood urea, serum creatinine, and 24 hours urinary albumin concentrations. Serum proteins and cholesterol were assessed at the end of treatment. Immunological studies were repeated when possible at the end of treatment. Response was termed as complete remission when the urine was free of albumin in three consecutive samples. Partial remission was when there was a reduction in proteinuria to + or ++ and the patient was free of oedema. No response was when there was no reduction in proteinuria. The patients were followed up for six months to two and a half years to assess their nephrotic state.

Results

There were 18 patients in relapse aged 1–12 years and 15 age matched controls. Tables 1 and 2 show the results of their immunological studies, respec-

Table 1 *Results of immunological study in 18 patients with minimal change nephrotic syndrome*

Case no	*Haemoglobin (g/l)*	*Serum albumin (g/dl)*	*Skin reactivity to dinitrochlorobenzene (grade)*	*Absolute mononuclear cell count (per mm³)*	*T cell count (per mm³)*	*B cell count (per mm³)*	*Phytohaemagglutinin index at 72 hours*	*IgG (mg/dl)*	*IgA (mg/dl)*	*IgM (mg/dl)*
1	114	2·0	2	5587	1898	509	3·8	500	84	176
2	128	1·4	2	3696	1182	813	2·1	400	124	190
3	136	1·0	3	1440	648	403	1·8	640	94	129
4	97	0·8	0	3744	1835	973	1·2	520	80	146
5	88	0·6	2	3328	1265	765	3·8	340	92	138
6	118	2·1	2	4575	1007	685	3·6	528	74	260
7	114	2·2	0	1760	387	176	3·9	400	74	140
8	118	0·6	2	4312	1207	1035	2·8	580	68	120
9	100	1·8	2	3432	1304	309	6·2	840	114	138
10	132	0·9	1	5928	512	1363	3·0	480	84	180
11	112	0·6	0	2331	652	303	2·6	460	100	136
12	125	2·5	0	3432	1510	412	1·2	380	64	120
13	104	1·9	2	3808	1312	609	3·2	840	110	143
14	110	1·5	2	2430	1966	437	3·6	480	48	120
15	100	3·4	1	2343	1267	398	2·4	620	72	141
16	120	1·2	0	3393	1243	238	4·9	720	96	154
17	114	1·5	1	4012	1387	422	3·6	840	84	126
18	108	1·8	0	6574	1942	1052	3·4	800	102	134

Table 2 *Results of immunological study in the 15 controls*

Case no	*Haemoglobin (g/l)*	*Serum albumin (g/dl)*	*Skin reactivity to dinitrochlorobenzene (grade)*	*Absolute mononuclear cell count (per mm³)*	*T cell count (per mm³)*	*B cell count (per mm³)*	*Phytohaemagglutinin index at 72 hours*	*IgG (mg/dl)*	*IgA (mg/dl)*	*IgM (mg/dl)*
1	142	3·9	3	4800	3168	1056	5·8	1100	101	143
2	135	3·2	2	6200	4216	1426	6·6	830	94	186
3	110	4·0	4	2920	1119	525	7·4	1349	97	96
4	132	3·8	3	8000	5120	1120	4·4	1760	101	89
5	118	3·9	4	11000	6490	1540	10·8	1400	120	181
6	112	3·5	Negative	12000	7440	2320	6·4	620	97	128
7	116	3·6	3	3800	2242	646	4·5	622	180	201
8	113	4·1	4	4800	3168	672	3·9	934	190	190
9	118	3·7	3	3200	1888	480	6·9	1760	120	136
10	111	3·4	4	4200	2394	672	3·8	1100	120	97
11	133	3·9	3	4000	2320	720	6·8	688	160	81
12	128	3·6	4	4600	2714	644	3·9	948	138	201
13	122	3·7	4	3800	2808	608	9·4	1100	180	96
14	147	3·9	3	2400	1512	432	4·8	800	101	138
15	120	3·3	3	3600	2374	792	7·8	990	124	134

tively. The statistical analysis of these results are as follows.

The absolute T lymphocyte count in patients with nephrotic syndrome had a mean (SD) value of 1387 (527). The mean (SD) value for the control group was 3265 (1808) ($p<0.001$) (Figure 1). The blastogenesis index to phytohaemagglutinin stimulation at 72 hours was 2·87 (1·17, SEM 0·284) in the patients while the mean (SD) value for the control group was 6·2 (2·01, SEM 0·539) ($p<0.001$) (Figure 2). The skin reactivity to dinitrochlorobenzene was grade 2 or less in 16 patients, while in the control group 13 had a response of grade 3 or 4. The mean (SD) value for the absolute B lymphocytes in nephrotics was 661 (391). In the control group it was 910 (512). The difference was not significant. The serum IgG concentrations in the nephrotic group was 576 mg% (SD 164, SEM 39) and in the controls was 1072 mg% (SD 348, SEM 93) ($p<0.001$).

The mean (SD, SEM) value for IgA concentrations in the patients with nephrotic syndrome was 87 (18, 4). In the controls it was 128 (32, 8). The difference was not significant. The IgM concentrations in the patients with nephrotic syndrome were

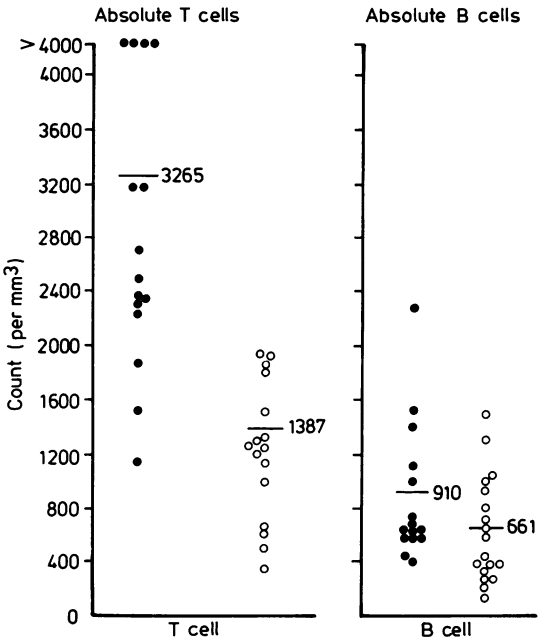

Fig. 1 *Absolute T and B cell lymphocyte counts in the group with minimal change nephrotic syndrome (n=18) and the control group (n=15).*

Patients with the syndrome are shown by ○, controls by ● ($p<0.001$ for absolute T cell count; p not significant for absolute B cell count). Values shown are the means.

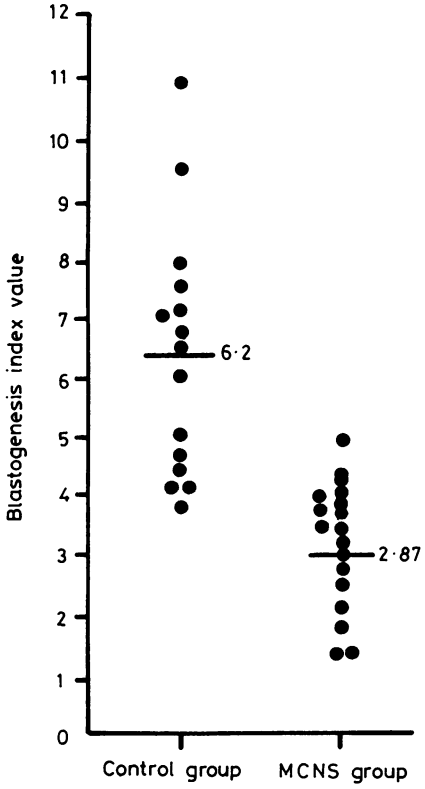

Fig. 2 *Blastogenesis index to phytohaemagglutinin stimulation at 72 hours in the group with minimal change nephrotic syndrome (MCNS) (n=18) and the control group (n=15).*

$p<0.001$. Value shown is the mean.

150 (SD 33, SEM 8). In the controls it was 140 (SD 41, SEM 11) (Figure 3). The serum complement concentrations in both the groups were within normal limits.

Results of treatment with levamisole. Fourteen patients with nephrotic syndrome were treated with levamisole. Their ages ranged from 1 to 12 years. Two were aged under 2, six between 2–5, and six over 5. There were eight boys and six girls. Six patients were seen in the first attack and had never received steroids, while out of the remaining eight cases responsive to steroids five infrequently relapsed and three frequently relapsed. One of those who frequently relapsed was also steroid dependent.

Of these 14 patients, six had complete remission, six had partial remission, and two did not respond to levamisole. The time taken to achieve remission varied from between four and 20 weeks of treat-

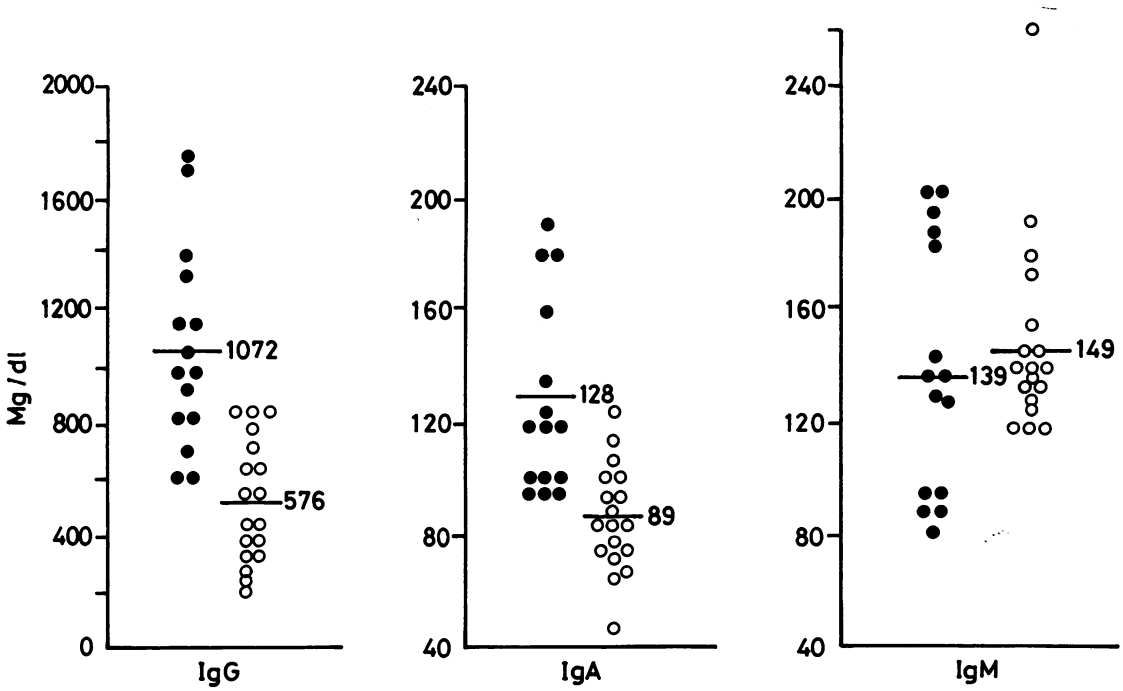

Fig. 3 *Serum IgA, IgG, and IgM concentrations in the group with minimal change nephrotic syndrome (n=18) and the control group (n=15).*

Patients with the syndrome are shown by ○, controls by ●. Values shown are the means.

ment, occurring slightly earlier (four to 12 weeks) in those who were on daily treatment. All the six patients aged over 5 showed a good response to treatment with levamisole; three went into complete remission and three into partial remission. Two patients who did not respond were aged under 5, one seen in first attack and the other who frequently relapsed. Of six patients seen in the first attack, four went into complete and one into partial remission. Of the five patients who infrequently relapsed, one went into complete remission and four into partial remission. Of these four in partial remission, two went immediately into complete remission after stopping treatment with levamisole. Of the three who frequently relapsed, one went into complete remission, one into partial remission, and one failed to respond. None of the 14 patients suffered from any severe infection during the six months. There was a reduced prevalence of infections most noticeable in six patients who had more than five episodes of severe infections in the previous year.

Side effects. Minor side effects were seen in those receiving daily treatment. These included vomiting in three cases, rash in one, and transient haematuria in two.

On follow up of 12 patients who showed an initial response to treatment with levamisole in the form of complete or partial remission, 6 months after stopping treatment, seven were in complete remission (five of six from the complete remission group, two of six from the partial remission group). Two were in partial remission, and three relapsed. Follow up of one to two years showed 7 of 12 in complete remission and 1 of 12 in partial remission.

Immunological studies after treatment with levamisole. T cell, B cell enumeration and blastogenesis index was repeated in two patients after remission induced by levamisole. In both these patients the blastogenesis index had increased. Immunoglobulins were repeated in seven patients. Six (five in complete and one in partial remission) showed increase in IgG concentration to more than twice the pretreatment values. In one patient in partial remission the IgG concentration was still low.

Discussion

Infections account for more than half the deaths due to minimal change nephrotic syndrome.[4] They are also responsible for the considerable morbidity seen in this disease. This increased vulnerability to infection can be accounted for by the depression in the immunological system as seen by the low cell count, depressed blastogenesis, poor skin reactivity to dinitrochlorobenzene, and low serum IgG concentrations.

Decreased response of lymphocytes to mitogens has been described in active nephrotic syndrome by several authors,[5–7] as has the poor skin reactivity to dinitrochlorobenzene.[5 8] Most authors, however, have reported the T cell to be numerically normal,[5 7] except in one other study by Tanphaichitr *et al* where the T cell counts were found to be low.[9] Low T cell counts were uniformly present in all our patients, only two of whom had received previous treatment with Endoxan, which is known to have a prolonged depressive effect on T cells.[10] Low serum IgG concentrations in relapse has been well documented and cannot be explained on urinary losses alone.[11 12]

It has not been established whether the depression in cell mediated immunity is a primary event or secondary to the hypoalbuminaemia, hyperlipidaemia, or zinc deficiency that coexists in these patients, as all these factors are known to depress cell mediated immunity.[13–15] It has been postulated that an abnormal immunogenic response to some unknown stimuli may be a primary event in patients with nephrotic syndrome, leading to suppression of T cell functions. This may lead to depressed cell mediated immunity as well as impairment of T cell dependent B cell activation accounting for the low concentrations of IgG in these patients. This same deviated immune response may be responsible for the production of an abnormal lymphokine with properties of increasing vascular permeability.[2] Such vascular permeability factors have been identified in the sera of patients with active nephrotic syndrome.[3]

Whether a cause or an effect, the immunological dysfunctions increase the risk of serious infections in these patients. The role of immunoregulatory drugs as opposed to immunosuppressive drugs in the management of these immunocompromised patients needs to be explored. Levamisole, used as an antihelminthic, has immunoregulatory properties. It is a non-specific stimulator of lymphocytes and has been found to increase both lymphocyte number and function. This action is particularly noticeable in immune depressed patients. It does not stimulate normal lymphocytes.[16] It has been successfully used in other immunologically mediated diseases such as rheumatoid arthritis, chronic active hepatitis, and recurrent skin infections.[16–18] Its successful use in minimal change nephrotic syndrome has been documented in a small number of children.[9]

We found that treatment with levamisole definitely reduced the number and severity of infections that in our experience often accompany or precede a relapse. This beneficial effect is probably due to its stimulant effect on cell mediated immunity. It has been used by some workers to treat certain varieties of recurrent and chronic infections with beneficial results.[16 17] Hence even in those nephrotics who have severe infections levamisole can be administered safely and probably to the advantage of the patient.

As opposed to this, steroids that act by depressing the T cell function increase the incidence and severity of infections, which may even be the cause of death in some instances.[4] In those patients presenting with infections treatment with steroids has to be postponed till the infection is well under control, thus delaying the beginning of definitive treatment. The effect of levamisole on the proteinuria, however, is slower and less dramatic compared with that of steroids.

In our study complete remission was achieved in six of 14 cases within 4–20 weeks of treatment. A total of 12 of 14 cases showed a definite reduction, however, in the proteinuria with improvement in the nephrotic state. Two of three patients who frequently relapsed benefited by this treatment. One went into complete remission, which was maintained for six months after stopping the drug; this child was steroid dependent, and this was the longest period free of disease that this child has had. The second patient who frequently relapsed remained free of both oedema and infection during the six months of treatment.

Several toxic effects, including agranulocytosis, have been described with the use of levamisole, especially in those receiving high daily doses.[16 19 20] In the schedule used by us there was no serious toxicity. Side effects are fairly common even with the recommended doses of steroids, while the dangerous side effects of prolonged treatment with steroids are well known.

Thus the role of levamisole in the management of minimal change nephrotic syndrome seems to be promising. The restoration of normal lymphocyte function by levamisole possibly helps to eliminate gradually the pathophysiological events leading to a nephrotic state. Further studies on the optimum dosage, regularity of administration, and duration of treatment require further investigation. Newer drugs with more specific actions on lymphocyte

subpopulations may have a better role in the management of nephrotic syndrome.

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Correspondence to Dr K P Mehta, The Nephrology Division, Bai Jerbai Wadia Hospital for Children, Institute of Child Health and Research Centre, Parel, Bombay 400 012, India.

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