

## CELL MEDIATED IMMUNITY IN POST-STREPTOCOCCAL GLOMERULONEPHRITIS

---

S. Rajajee  
P.R. Narayanan  
S.G.P. Moses  
N. Sundaravalli

### ABSTRACT

*Cell mediated immunity was assessed in 30 children with acute post streptococcal glomerulonephritis (APSGN) in parallel with 20 normal children and 15 children without nephritis who showed evidence of skin-sore  $\beta$ -Hemolytic streptococcal infection. Delayed cutaneous hypersensitivity to 2,4, dinitrochloro benzene (DNCB) was similar in the three groups. There were no significant differences in the proportion of early and total T-rosettes. Lymphocyte transformation response to phytohemagglutinin-P (PHA), purified protein derivative (PPD) and BCG was similar in them, indirect leucocyte migration inhibition response to PPD, streptokinase streptodornase (SK-SD), and group A  $\beta$ -Hemolytic T<sub>12</sub> streptococcal antigens were not significantly different in patients when compared to normal controls and streptococcal infection controls. Cell mediated immunity was normal in APSGN in children by all the parameters studied.*

**Key words:** Cell mediated immunity, Acute post-streptococcal glomerulonephritis.

The prevalence of APSGN has declined in the developed countries but it remains relatively common in tropical developing countries. Epidemics of APSGN following skin infection such as Impetigo or infected scabies have been reported from several parts of the world(1-3). At the Institute of Child Health, Madras, an average of 432 cases of APSGN are admitted per annum with an average of 35.8 per month, majority of the cases followed infected scabies or impetigo. The factors responsible for the high incidence are poor hygiene and over-crowding which predispose to recurrent scabies and impetigo. The role of undernutrition in predisposing to APSGN has to be assessed because of the depressive effect of undernutrition on cell mediated immunity(4,5).

The important role played by the humoral immune system in the pathogenesis of glomerulonephritis is well known while the involvement of cell mediated Immunity-is doubtful.

There is very little reported data on cell mediated immunity in APSGN in children.

The present study was undertaken to assess the cell mediated immunity (CMI) during the acute phase of APSGN, and this was compared to the CMI in skin infection (contact) controls and normal controls.

### Material and Methods

The study consisted of 3 groups of children.

(A) APSGN (30 children) during the acute phase.

The following criteria were taken to classify them:

- (1) Acute onset of oliguria and edema
- (2) Proteinuria and microscopic or gross hematuria

---

*From the Department of Pediatrics and Medicine, Madras Medical College and Department of Immunology, Tuberculosis Research Centre, Madras.*

- (3) Transient rise of blood pressure.
- (4) Other clinical features such as pulmonary edema, seizures or renal failure.
- (5) No past history of renal disease.
- (6) Evidence of previous streptococcal infection such as raised Anti-D Nase B levels and/or positive culture.
- (7) Low C<sub>3</sub> levels.
- (B) Skin infection (contact) controls (15 children) consisted of siblings of patients with skin sepsis but normal urinary findings, raised anti-D Nase B levels and/or positive culture and normal C<sub>3</sub> levels.
- (C) Normal children (20 children) were children of employees attending a medical centre, with no evidence of skin infection, normal urinary findings, anti-D Nase B levels and C<sub>3</sub> levels.

The nutritional status of the children was assessed after discharge, during follow up and graded according to ICMR reference standards.

Delayed cutaneous hypersensitivity to DNCB, (Koch-light Laboratories, U.K.) was tested by the method of Catalona *et al.* (6) but with the lower sensitising dose of 500 µg/ml because at the higher dose of 1000 µg/ml severe ulceration occurred(7).

#### *Lymphocyte separation*

Samples of peripheral blood were drawn from the children. Lymphocytes were isolated by Ficoll Hypaque density gradient centrifugation. The cells at the interface were collected, washed thrice in Hank's Balanced salt solution, the viability was approximately 95 % cells as revealed by the Trypan blue exclusion test.

#### *Cell surface markers*

T-cells were enumerated in two sepa-

rate determinations using the rapid rosette technique(8) as well as total sheep cell rosettes incubated at 4°C and read after 12 to 18 hours incubation(9).

#### *Lymphocyte transformation*

The lymphocytes were cultured in duplicates in 1 ml of RPMI 1640 supplemented with penicillin (100 µg/ml), streptomycin 100 µg/ml glutamine (300 µg/ml) and 0.1 ml autologous plasma in 24 well tissue culture plates (Laxbro) at a concentration of  $1 \times 10^6$  cells per ml. The cells were stimulated with 1 µg/ml phytohemagglutinin P (PHA-P-Wellcome Burroughs), 50 µg/ml purified protein derivative (PPD-preservative free Central Veterinary Lab., U.K.) and 50 µg/ml BCG (BCG Laboratories, Madras). Cultures were incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for 96 hours for PHA and 144 hours for PPD and BCG.

The proliferative response was measured by adding 10 uci of 3 H Thymidine (Sp Act 13000 MCi/Mol Babha Atomic Research Centre, Bombay) 16 hours before harvesting. At the time of harvest, 0.2 ml of lymphocyte cell suspension was transferred from each well of 24 well plates into 96 well plates in triplicates, subsequently harvesting was done with MASH II (Microbiological associates USA) and deposited on (Whatman) fibre glass paper. Paper discs were then transferred to biovials containing 1 ml of scintillation fluid and counted in a scintillation counter. Stimulation Index was calculated as follows:

$$\text{S.I.} = \frac{\text{CPM in stimulated cultures}}{\text{CPM in control cultures}}$$

Indirect leucocyte migration inhibition test.

*Streptococcal antigens*

Pure cultures of Group A  $\beta$ -hemolytic streptococcus T<sub>12</sub> which was isolated from a nephritic patient was used.

The bacterial suspension was killed by Beating at 70°C for 1 hour in a water bath, different wet weight concentrations were used, optimal concentration was found to be 1 mg/ml.

*Lymphokines*

Purified lymphocytes obtained from peripheral blood as described earlier were suspended in RPMI 1640 with penicillin 100  $\mu$ g/ml streptomycin 100  $\mu$ g/ml, glutamine 300  $\mu$ g/ml with 10% sterile horse serum at a final concentration of  $1 \times 10^6$  cells/ml. The cells were stimulated with 50  $\mu$ g/ml PPD, 200 units/ml SK-SD and 1 mg/ml streptococcal bacillary suspension with appropriate controls. The cultures were incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for 96 hours. After centrifugation, the supernatant was separated.

Peritoneal cells were collected from healthy guinea pigs after stimulation with liquid paraffin. The peritoneal cells were allowed to migrate in 12 well LMI plates (Laxbro) surrounded by lymphokines obtained in the presence of antigens and controls. The plates were incubated at 37°C for 18 hours, migration patterns were projected at fixed magnification and traced. The projected areas were measured by cutting out and weighing. The assay was set up in triplicates.

Migration Index (MI) =

$$\frac{\text{Area of migration in the presence of antigens}}{\text{Area of migration in controls}}$$

MI below 0.8 was taken as indication of positive reactors.

**Results**

*Table I* indicates the nutritional status of the patients and skin infection controls. Majority are normal weight for age or had mild undernutrition and the children in both the groups were in the same nutritional range.

Delayed cutaneous hypersensitivity to DNCB was positive in all the patients as well as skin infection and normal controls ranging from 4<sup>+</sup> to 1<sup>+</sup> (*Table II*).

The proportion of early T-rosettes was 22.4, 22.2 and 20.2% in patients, normal controls and skin infection controls respectively. There was also no significant difference in the proportion of total T-rosettes which was 58.6% in patients, 59.25% in normal controls and 57.4% in skin infection controls. This is indicated in *Table III*.

The mean values of the mitogenic response to PHA of patients, skin infection (contact) controls and normal controls are shown in *Table IV*. T-cell function as measured by the response to PHA in autologous plasma was normal in patients as compared to the response in skin infection controls and normal controls.

The lymphocyte response of patients to PPD and BCG was not significantly different from that of skin infection and normal controls (*Table IV*).

Similarly, in the indirect leucocyte migration inhibition test the mean values for migration index in response to PPD was not different in patients when compared to controls (*Table V*).

The responsiveness of the lymphocytes to SK-SD was similar in patients, skin infection controls and normal controls (*Table V*).

**TABLE I** – *Nutritional status of children with APSGN*

Subjects	No.	Grading of nutrition			
		Normal	1	2	3
1. Acute nephritis	135	72	50	13	–
2. Skin infection (controls)	32	14	14	4	–
3. Normal (controls)	20	12	6	2	–

Classified using ICMR Reference Standards  
 Upto 80% of Standard for weight -Normal  
 70–80% -Mild undernutrition  
 60–70% -Moderate undernutrition  
 Below 60% -Severe undernutrition

**TABLE II** – *Delayed cutaneous hypersensitivity to DNCB in APSGN sensitisation dose 500 µg/0.1 ml challenge dose 50 µg/0.1 ml*

Group	No. of subjects	DNCB positive				DNCB negative
		4 <sup>+</sup>	3 <sup>+</sup>	2 <sup>+</sup>	1 <sup>+</sup>	
Acute Nephritis	60	20	30	6	4	Nil
Normal (control)	50	17	31	2	–	Nil
Skin infection (control)	15	5	9	1	–	Nil

**TABLE III** – *Comparison of the peripheral blood early and total lymphocytes in ASPGN and controls*

Group	No.	% T-rosettes	
		Early	Total
Acute nephritis	30	22.4 ± 6.7 (8-36)	58.6 ± 8.10 (44-72)
Normal (controls)	20	22.2 ± 4.5 (13-30)	59.25 ± 6.2 (46-70)
Skin infection (controls)	5	20.2 ± 4.14 (16-27)	57.4 ± 6.6 (47-64)

**TABLE IV** – *Lymphocyte transformation response to PHA, PPD and BCG in APSGN and controls*

Group	Stimulation index mean and SD		
	PHA	PPD	BCG
Acute nephritis	43.9 ± 31.3 (26)*	4.6 ± 7.3 (26)	7.6 ± 16 (23)
Normal (controls)	41.7 ± 26.1 (15)	5.7 ± 11.8 (13)	2.8 ± 1.3 (11)
Skin infection (controls)	48.6 ± 7.3 (5)	2.10 ± 2.1 (5)	4.01 ± 1.5 (5)

\*Number of subjects.

**TABLE V** – *Indirect LMI response to streptococcal antigens SK/SD and PPD in APSGN and controls*

Group	Migration index mean and SD		
	Streptococcal antigens	SK-SD	PPD
Acute nephritis	0.76 ± 0.6 (32)*	0.68 ± 0.5 (15)	0.76 ± 0.7 (15)
Normal (controls)	0.75 ± 0.6 (15)	0.65 ± 0.3 (12)	0.45 ± 0.3 (14)
Skin infection (controls)	0.93 ± 0.4 (10)	0.69 ± 0.5 (10)	–

\*Number of subjects.

Response to the streptococcal bacillary suspension showed no significant difference in the three groups. The mean migration index in response to the whole cell is indicated in *Table V*.

### Discussion

The role of undernutrition with its depressive effect on CMI was assessed in APSGN. The patients and skin infection controls were in the same nutritional range and most of the patients were nor-

mal weight for age or had mild under-nutrition.

Delayed cutaneous hypersensitivity to DNCB was normal in our patients. The proportions of early and total T-rosettes were normal in patients and skin infection control in our study. Williams *et al.* in 1977(10) in their study on surface markers in APSGN reported similar findings. Subsequently, in 1981(11) they reported that T-cells were decreased during the acute stage when compared to skin infection controls, but was not different from

normal controls. This finding of low T-cells during acute phase was thought to reflect hypo-reactivity to streptococcal antigens. Apart from finding normal T-cells numbers in patients and skin infection controls, we also found good response to mitogen PHA and heterologous antigens PPD and BCG in all the 3 groups of children in the lymphocyte transformation test. Bhat *et al.* (12) also reported normal lymphocyte transformation response to mitogens in their study on adult acute nephritis.

Response to SK-SD and PPD was present in majority of the patients and controls in the indirect leucocyte migration inhibition test. There was also no significant difference in the response to the bacillary suspension of T<sub>12</sub> Group A  $\beta$ -Hemolytic streptococcus between the patients, skin infection and normal controls. Regarding response to streptococcal membrane antigens, Bhat *et al.* (12,13) and Baldwin *et al.* (14) reported that lymphocyte transformation was depressed in patients with acute nephritis. In both these studies, the patients were adults with progressive disease. The prognosis is different in children when compared to adults and this could account for the different response in children (15).

The normal controls in our study also responded well to SK-SD and streptococcal antigens. This was probably because most "normal" children belonging to the low socio-economic strata are exposed to scabies and impetigo infected by various types of streptococci. Besides, the bacillary suspension was used and hence there was multiple antigenic stimuli.

In conclusion, cell mediated immunity was normal in patients and skin infection controls by all the parameters studied and not significantly different from normal controls. Purified streptococcal antigen

could probably bring out a difference in response between controls and patients.

## REFERENCES

1. Kaplan EL, Anthony BF, Chapman SS. Epidemic acute glomerulonephritis associated with type 49 streptococcalpyoderma. *Am J Med* 1970, 48: 9.
2. Dillon HC, Reeves Mary S, Maxted WR. Acute glomerulonephritis following skin infection due to strep M Type 2. *Lancet* 1968, i: 533.
3. White R. Diseases of the Kidney and Urinary Tract. In: Jelliffe and Morley (eds) *Diseases of Children in Subtropics and Tropics*. Arnold Publications 1981, pp 433-434.
4. Vinodhini Reddy, Bhaskaram C, Raghuramurlu N. Immunological responses in malnourished children. *Indian Pediatr* 1977, 14: 255.
5. Chandra RK. Immunocompetence as a functional index of nutritional status. *British Med Bull* 1981, 37: 89.
6. Catalona WJ, Taylor PT, Robson AS, Cheetein PB. DNCB contact sensitisation. *New Eng J Med* 1972, 286: 399-402.
7. Sarala Rajajee. Delayed cutaneous hypersensitivity to DNCB in iron deficiency anemia presented at the Indian Academy of Pediatrics Conference at Madras, Feb 1983.
8. Wybran J, Shireen Chantler, Hugh Frudenberg. Isolation of Normal T-cells in chronic lymphatic leukemia. *Lancet* 1973, i: 126.
9. Jondal M, Helm G, Wigzell H. Surface markers of T and B lymphocytes I. A large population of lymphocytes forming non-immune rosettes with sheep blood cells. *J Exp Med* 1972, 136: 207.
10. Williams RC (Jr), Zabriskie JB, Mabrose F, Hassaballa F, Godvin ZH. Lymphocyte surface markers in acute rheumatic fever and post streptococcal acute glomerulonephritis. *Clin and Exp Immunol* 1977, 27: 135-142.
11. Williams RC (Jr), Van De Rijn I, Reid H, Poon T, King Zabriskie JB. Lymphocyte cell sub-populations during acute post-streptococcal glomerulonephritis; cell surface antigens and binding of streptococcal membrane antigens and C-reactive protein. *Clin Exp Immunol* 1981, 46: 397-405
12. Bhatt JG, Gombos EA, Baldwin DS. Depressed cellular immune response to

- streptococcal antigens in post-streptococcal glomerulonephritis. *Chin Immunopathol* 1977, 7: 230.
13. Bhat JG, Gombos EA and Baldwin DS. Depressed CMI in response to streptococcal antigens in post-streptococcal glomerulonephritis. *Clin Immunol Immunopathol* 1980, 16: 48.
14. Baldwin DS, Robert GS, Gloria RG, Gluck MC, Feiner HD. Natural history of post streptococcal glomerulonephritis. In: Read SE, Zabriskie JB (eds) *Streptococcal Diseases and the Immune Response*. New York, Academic Press Inc. 1980, pp 563-579
15. Rammelkamp CH. Acute Post-streptococcal glomerulonephritis. In: Read SE, Zabriskie JB (eds) *Streptococcal Diseases and the Immune Response*. New York, Academic Press Inc 1980, pp 43-51.
- 

---

## NOTES & NEWS

---

Dr. N.D. Datta Banik, Deputy Director General, Indian Council of Medical Research has been elected as member of the American Academy of Pediatrics