·论著(译文)·

Immune Function of T- lymphocyte and Erythocyte in Children with Febrile Convulsion

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Abstract: Objective To study the immune function of T-lymphocyte and erythrocyte in the peripheral blood of children with febrile convulsion. **Methods** Eighty-two children with typical febrile convulsion, 40 children with acute upper respiratory tract infection (URI) and 40 normal children were enrolled. The proliferation reaction of T-lymphocyte to PHA, distribution of T-lymphocyte phenotype subsets, expression of activation markers CD25 (IL-2R) and HLA-DR, and level of γ-interferon induced by PHA were assayed. Erythrocyte immune function was simultaneously measured by rosette formation rates of RBC-C3bR and RBC-IC. **Results** The counts per minute (CPM) and stimulation index (SI), the measurements expressing T-lymphocyte proliferation assay in simple febrile convulsion (SFC) children, were 5 609.4 ±3 587.4 and 20.5 ±15.6, and 2 817.3 ±2 422.8 and 11.0 ±8.4 in complex febrile convulsion (CFC) children. They were significantly lower than those in the normal controls (20 305.9 ±12 810.3 and 69.2 ±45.2) and in the URI group (9 785.2 ±7 509.8 and 44.5 ±39.8) ($P < 0.05$). The CD3, CD4 and CD4/CD8 ratio in the SFC children were (40.0 ±8.2) %, (26.1 ±9.0) % and 1.1 ±0.4 and (932.8 ±6.9) %, (17.8 ±4.9) % and 0.8 ±0.1 in the CFC children. They were all significantly lower than those in the normal controls [(64.1 ±6.7) %, (47.7 ±5.5) % and 1.9 ±0.8] and in the URI group [(63.0 ±9.3), (42.4 ±8.2) % and 1.6 ±0.4] ($P < 0.01$). The expression rates of CD25 (IL-2R) and HLA-DR antigen in the spontaneous condition in the SFC children were (8.9 ±3.6) % and (16.2 ±5.6) % and (6.3 ±1.9) % and (12.4 ±3.4) % in the CFS children. They were lower than those in the normal controls [(12.8 ±2.5) % and (20.2 ±5.2) %]and in the URI group [(15.0 ±3.0) % and (20.5 ±2.8) %] ($P < 0.01$) and there were also differences in the SFC children and in the CFS children ($P < 0.05$). After PHA induction, the expression rates of CD25 (IL-2R) and HLA-DR antigen in the SFC children were (57.0 ±5.1) and (57.8 ±6.0) and (53.0 ±12.0) % and (54.7 ±9.7) % in the CFC children. They were significantly lower than those in the normal controls [(65.7 ±5.7) % and (68.8 ±6.2) %] ($P < 0.05$) and in the URI group [(64.3 ±6.4) % and (67.1 ±8.6) %] ($P < 0.01$). The γ-IFN level of PBMC induced by PHA in the SFC and CFC children [(1.80 ±0.4) and (1.6 ± 0.1) ng/ml] was significantly lower than that in the normal controls [(2.4 ±0.9) ng/ml] ($P < 0.05$). No difference was found compared with the URI group. The rate of rosette formation of RBC-C3b in the SFC children was (9.1 ±4.9) % and it was significantly lower than that in the normal controls and the URI group [(15.8 ±5.7) % and (13.5 ±5.1) %] ($P < 0.01$), but there was no difference in the SFC children, the normal controls and the URI group. The rate of rosette formation of RBC-IC in the SFC children and the CFC children [(3.0 ±1.0) and (2.6 ±0.7) %] was significantly lower than that in the normal controls [(3.7 ±1.3) %] and the URI group [(3.9 ±1.4) %] ($P < 0.05$). **Conclusions** Both T-lymphocyte immune function and erythrocyte immune function in the children with FC were significantly impaired. The impair was more severe in the CFC children than that in the SFC children.

Key words: Febrile Convulsion; T-lymphocyte, RBC Immune function; Child

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The nervous system is closely related to the immune system. Although the serum IgA level in children with febrile convulsion (FC) is often reduced, alterations of cell-mediated immune response in FC remains to be studied in detail. The objective of this study was to find out the immuno-functional status of

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T-lymphocyte and erythrocyte in the peripheral blood of children with FC and to establish a theoretical basis of proper administration of immuno-regulatory agents to these children for prevention and treatment.

1 Methods

1.1 Subjects

Eighty-two children (58 boys and 24 girls with an average age of 2.5 years) with FC secondary to upper respiratory infection and admitted to Jiangxi Children's Hospital between January, 1997 and December, 1999 were enrolled in this study. They all met the FC diagnosis criteria[1] proposed at the First National Symposium of Pediatric Neurology of China and were excluded for epilepsy by electroencephalograms. They were further divided into the simple febrile convulsion (SFC) subgroup and the complex febrile convulsion (CFC) subgroup according to the definitions used by Verity[2]. Those whose convulsions lasted longer than 15 minutes and were focal or multiple (more than one convulsion per episode of fever) belonged to the CFC subgroup (n = 41) and the others (n = 41) formed the SFC subgroup. Blood samples were drawn within 24 h after the onset of convulsion.

In order to exclude the influence of infection and fever on erythocyte immune function, 40 URI children (26 boys and 14 girls with an average age of 2.6 years) admitted in the same period were studied for comparison. Blood samples were drawn on the next morning after admission. Forty normal children (28 boys and 12 girls with an average age of 2.8 years) without any disease for at least one month before the study were used as the normal control group.

No subject in this study received any immuno-inhibitory or immuno-enhancing agent at least in one month before the study.

1.2 Agents and equipment

The ^{3}H-TdR kits used in the study were obtained from Shanghai Atomic Energy Research Institute; Scintilator, Beckman, USA. The McAb and APAAP, McAb of CD25, McAb of HLA-DR and APAAP kits were provided by the Military Medical Science Academy, Beijing. γ-IFN kits were provided by the Second Military Medical University, Shanghai; ELSA Analyzer Microp late Reader Modle 3550, Bio-Rad.

1.3 Methods

1.3.1 Assay of T-cell proliferation T-cell proliferative reaction to PHA was tested on blood samples by using the micro-whole-blood ^{3}H-thymidine assay[3]. The results were expressed in the counts per minute (CPM) and stimulation index (SI).

1.3.2 Assay of T-cell subsets The distribution of T-lymphocyte phenotype subsets (rates of CD3, CD4, CD8 and CD4/CD8 ratio) was determined by using the monoclonal antibody (McAb) APAAP assay.

1.3.3 Assay of the expression of CD25 (IL-2R) marker and HLA-DR marker The expression rates of the two markers were determined under a spontaneous condition and after PHA stimulation by using the McAb APAAP assay.

1.3.4 Assay γ-IFN level The level of γ-IFN of PBMC induced by PHA was measured by ABC-ELISA.

1.3.5 Assay for immuno-absorbent function of RBC The function of immuno-absorbent capacity of RBC was tested by rosette formation rates of RBC-C3bR (RBC-C3bRR) and of RBC-IC (RBC-ICR) using Guo's[4] modified RBC-Yeast Rosette assay (yeast from General Yeast Plant, Shanghai).

1.4 Statistical analysis

All statistical analyses were performed using POMS-5 of the "Program of Medical Science Analysis" software package. All values were presented as means ± s ($\bar{x}$ ± s). Differences between the two groups were analyzed by using the single factor analysis of variance and F test. Differences between the two groups were tested using q test and the P value was accounted.

2 Results

The proliferative reactions of T-lymphocyte of each group are shown in Table 1. The proliferative reactions in terms of CPM in the SFC and CFC children were all significantly lower than those in the normal group and URI group respectively (P <

0.05). The values of SI of the SFC and CFC children were also significantly lower than those in the normal group and URI group respectively ($P < 0.01$). The values of CPM and SI were profoundly decreased in the CFC children than those in the SFC children ($P < 0.05$) (Table 1).

Table 1 Comparison of reaction of T-lymphocyte proliferation and T-lymphocyte subsets distribution in 3 groups ($\bar{x} \pm s$)

Groups	Numbers of Children	CPM	SI	CD3(%)	CD4(%)	CD4/CD8
Control	40	20 305.9 ±12 810.3	69.2 ±45.5	64.1 ±6.7	47.7 ±5.5	1.9 ±0.3
URI	40	9 785.2 ±7 509.8	44.5 ±39.8	63.0 ±9.3	42.4 ±8.2	1.6 ±0.4
FC						
SFC	41	5 609.4 ±3 587.4^{a,b}	20.5 ±15.6^{a,b}	40.0 ±8.2^{a,b}	26.1 ±9.0^{a,b}	1.1 ±0.4^{a,b}
CFC	41	2 817.3 ±2 422.8^{a,b,c}	11.0 ±8.4^{a,b,c}	32.8 ±6.9^{a,b,c}	17.8 ±4.9^{a,b,c}	0.8 ±0.1^{a,b,c}

a vs control group, $P < 0.05$; b vs URI group, $P < 0.05$; c vs SFC group, $P < 0.05$

The distribution of T-lymphocyte phenotype subsets and CD4/CD8 ratio are also shown in Table 1. The values of CD3, CD4 and CD4/CD8 ratio were significantly lower in the FC group than those in the normal control group and URI group and the ratio of CD4/CD8 in 65% (54/82) FC children were reversed. CD3 cells, CD4 cells and CD4/CD8 ratio in the peripheral blood of the SFC and CFC children were significantly lower than those in the normal children and URI group ($P < 0.01$). CD3 cells, CD4 cells and CD4/CD8 ratio in the CFC children were also significantly lower than those in the normal group and URI group ($P < 0.01$). It is obvious that the percentages of CD3 cells and CD4 cells and CD4/CD8 ratio in the CFC children were all further reduced than those in the SFC children ($P < 0.01$).

The expression rates of CD25 (IL-2R) antigen and of HLA-DR antigen before and after PHA induction in each group are shown in Table 2. The two expressions before PHA induction in the SFC children and also in the CFC children were reduced than those in the normal group ($P < 0.01$) and URI group ($P < 0.01$). After PHA induction, the expression rates of CD25 antigen and HLA-DR antigen in the two subgroups became higher than before ($P < 0.01$), but both CD25 antigen expression and HLA-DR antigen expression in the SFC and CFC children still remained less than those in the normal children ($P < 0.01$) and also less than those in the URI group ($P < 0.01$). The reduction of both CD25 antigen expression and HLA-DR antigen expression under the spontaneous condition were more profound in the CFC children than those in the SFC children ($P < 0.01, 0.05$), but there was no significant difference between these two subgroups following PHA induction ($P > 0.05$).

Table 2 Comparison of expression of T-lymphocyte activation markers CD25 and HLA-DR before and after PHA introduction ($\bar{x} \pm s$, %)

Groups	Number of Children	Before PHA		Number of Children	After PHA	
		CD25	HLA-DR		CD25	HLA-DR
Control	22	12.8 ±2.5	20.2 ±5.2	12	65.7 ±5.7	68.8 ±6.2
URI	22	15.0 ±3.0	20.5 ±2.8	12	64.3 ±6.4	67.1 ±8.6
FC						
SFC	26	8.9 ±3.6^{a,b}	16.2 ±5.6^{a,b}	12^{a,b}	57.0 ±5.1^{c,f,g}	57.8 ±6.0^{c,f,g}
CFC	14	6.3 ±1.9^{a,b,e}	12.4 ±3.4^{a,b,e}	10	53.0 ±12.0^{d,f,g}	54.7 ±9.7^{d,f,g}

a vs control group, $P < 0.01$; b vs URI group, $P < 0.01$; c vs SFC group before PHA, $P < 0.01$; d vs CFC group before PHA, $P < 0.01$; e vs SFC group before PHA, $P < 0.05$; f vs the control group after PHA, $P < 0.05$; g vs URI group after PHA, $P < 0.01$

The γ-IFN levels of PBMC after PHA induction in the 3 groups are shown in Table 3. Compared with those of the normal children, the γ-IFN levels induced were less in both the SFC children ($P < 0.05$) and the CFC children ($P < 0.01$), but when compared with the URI group, there was no signifi-

cant difference with the SFC subgroup or CFC subgroup ($P > 0.05$). The depression of γ-IFN levels was more profound in the CFC children than that in the SFC children ($P < 0.05$) (Table 3).

Table 3 Comparison of the levels of γ-IFN induced by PHA in the 3 groups ($\bar{x} \pm s$, ng/ml)

Group	Number of Children	γ-IFN (ng/ml)
Control	18	2.4 ±0.9
URI	18	1.8 ±0.7
FC		
SFC	21	1.8 ±0.4[a]
CFC	17	1.6 ±0.1[a,b]

a vs control group, $P < 0.05$; b vs SFC group, $P < 0.05$

Rosette formation rates of RBC immuno-absorbent capacity in the 3 groups are shown in Table 4. Although there was no significant difference between the rosette formation rate of RBC-C3bR in the SFC children and those in both the normal group and the URI group ($P > 0.05$), the rosette formation rate of RBC-IC in the SFC children was lower than that in the normal group and URI group ($q = 4.32$, 3.07, $P < 0.01$, 0.05). The rosette formation rates of both RBC-C3bR and RBC-IC in the CFC children were all significantly less than those in the normal group and URI group ($q = 6.01$, 4.23, $P < 0.01$; $q = 5.66$, 4.45, $P < 0.01$). Although there was no significant difference in the rosette formation rate of RBC-IC between the CFC children and SFC children ($P > 0.05$), the RBC-C3bR rosette formation rate in the former was lower than that in the latter ($P < 0.01$).

Table 4 Comparison of the rate of forming RBC-C3bR and BRC-IC ($\bar{x} \pm s$, %)

Group	Number	RBC-C3bRR	RBC-ICR
Control	24	15.8 ±5.7	3.7 ±1.3
URI	32	13.5 ±5.1	3.9 ±1.4
FC			
SFC	27	13.1 ±5.3	3.0 ±1.0[a,b]
CFC	20	9.1 ±4.4[a,b,c]	2.6 ±0.7[a,b]

a vs control group, $P < 0.05$; b vs URI group, $P < 0.05$; c vs SFC group, $P < 0.05$

3 Discussion

It has been proved that the nervous system, endocrine system and immune system all together form a complete network. They are inter-linked and inter-regulated through the cytokine release, surface-protein expression, common signal molecule sharing and common receptor sharing. Alteration of one of the three systems would lead to the alterations of the other two. FC cases are relatively commonly seen in the pediatric emergency clinic and has the subsequent risks of epilepsy and mental deficiency in later life. The real etiology of this syndrome is not yet very clear, although the involvement of syndrome specific genes[5] and incomplete maturation of the nervous system or immune system defect have been proposed. Hafez[6] (1987) reported that the means of serum IgA and spontaneous E-rosette formation in the FC children were significantly lower than the normal controls ($P < 0.01$). Montelli[7] (1997) reported that 64% of the FC children presented an increased number of CD8 cells and a low CD4/CD8 ratio, and an impaired proliferative response of T-lymphocyte to PHA was also seen. However, the cell-mediated immune function status in FC children still remains to be studied in detail. The data of T-lymphocyte proliferative reaction to PHA induction, distribution of T-lymphocyte phenotype subsets, expression rates of CD25 marker and HLA-DR marker both under a spontaneous condition and after PHA induction, and γ-IFN levels of PBMC induced by PHA in FC children in our present study all evidenced the existence of T-lymphocyte immune function impairment in FC children and especially more profound in CFC ones. Moreover, each one of our data was in accordance with the rest. The proliferation level of T-lymphocyte is proportional to it's function level. A low level of T-lymphocyte proliferation indicates that the lymphocyte function is under depression due to the effect of immunogen. The expression levels of CD25 (IL-2R) and HLA-DR indicate the activation level of CD4 T-cells. The proliferation of T-lymphocyte needs the expression of both CD25 (IL-2R) antigen and HLA-DR antigen. The reduction datum of proliferative reaction of lymphocyte is in accordance with the reduction data of the expression of both CD25 antigen and HLA-DR antigen. The common effect of the two reductions would doubtlessly lead to the reduction of both the number

and the function of CD4 which would then result in the impairment of γ-IFN release from CD4 cells. The reduction of γ-IFN would in turn lead to the reduction of the expression of Tac antigen gene and the reduction of the transcription of DR and thus further impair the activation of both T-lymphocyte and B-lymphocyte. The reduction of CD4 in the FC children in our study was very significant, but the increase of CD8 was not apparent. The significant reduction of CD4/CD8 ratio was mainly due to the relative increase of CD8 and this might be caused by the result of simultaneous Ts increase and Tc decrease in CD8 cells.

The immune function of RBC is closely related to that of lymphocyte. LFA-3, the surface antigen of RBC, is able to combine with the CD2 molecule of lymphocyte and thus not only promotes IL-2R expression, γ-IFN secretion, differentiation and proliferation of T-lymphocyte, but also simultaneously promotes the differentiation and proliferation of B-cells[2]. We also found that the immuno-absorbent function of RBC in the FC children was also impaired in our present study. Both the rosette formation rate of RBC-C3b (RBC-C3bR) and that of RBC-IC (RBC-ICR) in the FC children were depressed compared with the rates in the normal children and this depression was especially more profound in the CFC group. Therefore, we agree that the depression of RBC immune function might suggest the existence, process and prognosis of some related nervous system diseases to a certain degree[9]. Due to the complete network formed by the immune system, nervous system and endocrine system, we do think that the immunal alterations of lymphocyte or/and those of erythrocyte would lead to the disturbance of the related cytokine levels in the blood and the brain and thus result in the impairment of equilibrium between excitatory mediators and inhibitory mediators of the brain cells and in turn alter the excitability of the brain cells. In addition, the presence of fever would enhance the excitability of the brain cells, especially when the nervous system has not yet been well developed. The final outcome of the alterations would cause clinical convulsions. Convulsions would further lead to brain anoxia and impairment and thus form a pernicious cycle of convulsion fits.

Therefore, we think that it is reasonable to use immuno-regulatory agents for FC children, especially for CFC children in the prevention and treatment of FC.

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