

# Interleukin-2 in relation to T cell subpopulations in rheumatic heart disease

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## Abstract

**Interleukin-2 (IL-2) and T cell subpopulations were evaluated in children with rheumatic heart disease (RHD). Three groups were included: 13 patients with active RHD, 12 with non-active RHD, and 14 control children. Serum IL-2 and T cell subpopulations were measured by radioimmunoassay and monoclonal antibodies respectively.**

Patients with active RHD showed a significant increase in IL-2 concentrations and helper:suppressor (H:S) ratio compared with controls with a mean (SEM) IL-2 of 3.48 (0.62) v 1.26 (0.16) U/ml and H:S ratio 2.31 (0.14) v 1.66 (0.04). There was a significant decrease in T suppressor (CD8+) and pan T (CD3+) cells compared with controls with a mean (SEM) for CD8+ of 23.75 (1.19) v 32.23 (0.56)% and CD3+ of 79.55 (0.94) v 85.00 (0.11)%.

Patients with non-active RHD showed a significant decrease only in the CD3+ cells (78.20 (0.20)%) when compared with controls. A deficiency of CD3+ cells is a constant finding in patients with RHD, whether the disease is active or not.

There was a significant increase in IL-2 concentration with a significant decrease in CD8+ cells in patients with active RHD in comparison with the non-active group (mean (SEM) IL-2 of 3.48 (0.62) v 1.85 (0.24) U/ml and CD8+ of 23.75 (1.19) v 28.83 (1.91)%). Thus an increase in IL-2 and a decrease in CD8+ cells may be related to rheumatic activity. T helper (CD4+) cells did not differ significantly between groups.

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Interleukin-2 (IL-2) is a major lymphokine secreted by T cells after their stimulation with a mitogen or allergen.<sup>1</sup> One of the cellular events

of immune reaction is secretion of IL-2. This IL-2 activates killer cells and supports growth of T and B cells via binding to specific surface receptors. Altered immune response to streptococcal antigens is implicated in the pathogenesis of rheumatic fever and rheumatic heart disease (RHD).<sup>2</sup> This altered immune response was mentioned as a diminished blastogenesis, increased leucocyte migration inhibition, and increased natural killer cell cytotoxicity.<sup>3,4</sup> Because of the importance of cytokines in the events of the immune reaction in various rheumatic diseases, we studied serum concentrations of IL-2 and T cell subpopulations in patients with active and non-active RHD.

## Patients and methods

Three groups of children were studied. (1) Thirteen patients had active RHD and all met the revised Duckett-Jones criteria for diagnosis of active rheumatic fever.<sup>5</sup> They were aged 8-12 years (mean (SE) 9.2 (0.1) years) and all had an old valvular lesion. (2) Twelve patients had non-active RHD. They were aged 8-15 years (mean (SE) 11.6 (0.3) years) and had a mitral valve lesion (diagnosed by echocardiography) and a history of acute rheumatic fever according to revised Duckett-Jones criteria<sup>5</sup> six months or more before the study. They had no evidence of heart failure or rheumatic activity. (3) Fourteen healthy children were selected as a control group. They were aged 7-14 years (mean (SE) 10.4 (0.4) years). None of these children had a history of rheumatic fever or chronic or repeated tonsillitis, there were no valvular lesions, and there was a normal erythrocyte sedimentation rate. The characteristics of the subjects are shown in table 1.

Blood samples for the measurement of IL-2 and T cell subpopulations were taken before the start of any treatment.

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Table 1 Characteristics of patients and control subjects

	Non-active RHD (n=12)	Active RHD (n=13)	Controls (n=14)
Mean (SE) age in years	11.5 (0.3)	9.2 (0.1)	10.4 (0.4)
Sex	9M:3F	5M:8F	10M:4F
Valve abnormality	Mitral valve only (n=11); mitral with aortic (n=2)	Mitral with tricuspid (n=3); mitral with aortic (n=2); mitral valve only (n=7)	No
Associated problems	No	Heart failure (n=6); arthritis (n=5); carditis and arthritis (n=1)	No
Mean (SE) antistreptolysin-O titre (Todd's units)	200.0 (19.8)	457.0 (149.1)	—
Mean (SE) total leucocyte count ( $\times 10^9$ cells/l)	6.3 (0.4)	8.5 (1.2) <sup>*</sup>	5.0 (0.6)
C reactive protein	Negative	Positive	Negative

\*p<0.05. There was a significant increase in total leucocyte count in active RHD in comparison with controls.

**Table 2** T cell subsets in active and non-active RHD in comparison with control subjects. Data are mean (SEM)

	Active RHD (n=13)	Non-active RHD (n=12)	Controls (n=14)
CD3+ (pan T) cells in %	79.55 (0.94)*	78.20 (0.20)†	85.00 (1.11)
CD4+ (T helper) cells in %	53.95 (0.96)	48.00 (2.74)	53.65 (0.70)
CD8+ (T suppressor) cells in %	23.75 (1.19)†	28.83 (1.91)	32.23 (0.56)
H:S ratio	2.31 (0.14)†	1.71 (0.16)	1.66 (0.04)

\*p<0.01; †p<0.001.

There was a significant decrease in CD3+ and CD8+ in active RHD in comparison with controls. There was no significant change in CD4+ in active and non-active RHD. Non-active RHD showed a significant decrease in CD3+ only. There was a significant increase in H:S ratio in active RHD in comparison with controls.

#### ASSAY OF IL-2

IL-2 was measured using a radioimmunoassay kit with radioactive iodine 125 supplied by Medgenix (Institut des Radio Elements, Fleurus, Belgium) according to radioimmunoassay principle.<sup>6</sup>

#### DETERMINATION OF T CELL SUBPOPULATION<sup>7</sup>

T cell subsets were determined by using fluoro-chrome conjugated reagents for T cell typing (Clonab monoclonal antibodies Biotest Diagnostics). Lymphocytes were separated from peripheral venous blood by Ficoll Hypaque gradient technique,<sup>8</sup> and adjusted to a concentration of 5 million/ml suspended in Hanks' medium. An aliquot of 100 µl of lymphocyte suspension was incubated with 200 µl of 1:20 prediluted and fluorescein isothiocyanate labelled Clonab antibody (CD3+, CD4+, and CD8+) at 4°C for 30 minutes. This was washed twice with cold phosphate buffer saline (PBS), the pellet resuspended in 100 µl of PBS, and counted using fluorescent microscopy.

#### STATISTICAL METHODS

Our data were analysed by the two tailed *t* test for independent samples.<sup>9</sup> All the data are expressed as mean (SEM).

#### Results

Table 2 shows the T cell subsets in the children with RHD in comparison with controls. There was a significant decrease in CD8+ and CD3+ cells and significant increase in the helper: suppressor (H:S) ratio in children with active RHD when compared with the control group (p<0.001, 0.01, 0.001 respectively); CD4+ cells showed a non-significant change in those patients. Patients with non-active RHD showed a significant decrease in CD3+ cells; this reflects the persistent decrease in CD3+ in patients with RHD whether the disease was active or non-active.

Table 3 shows IL-2 concentrations in RHD (active and non-active) in comparison with controls. We found a significant increase of IL-2 in active RHD in comparison with controls.

**Table 3** IL-2 concentrations in active and non-active RHD in comparison with control subjects. Data are mean (SEM)

	Active RHD (n=13)	Non-active RHD (n=12)	Controls (n=14)
IL-2 (U/ml)	3.48 (0.62)*	1.85 (0.24)	1.257 (0.156)

\*p<0.01. There was a significant increase in IL-2 in active RHD in comparison with controls.

#### Discussion

Generation of the cellular immune response depends upon two signals. The first one is the interaction of antigen or mitogen with macrophages; this results in their activation and the secretion of a soluble product called lymphocyte activating factor, now termed interleukin-1 (IL-1). This IL-1 is responsible for initiation of the second signal which results in two responses. (A) Activation of the T cell subpopulation, mainly T helper cells (CD4+), to express specific receptors for another soluble factor called T cell growth factor or IL-2. (B) The combination of the antigen with IL-1 and this induces the subset of T cells to secrete IL-2. The IL-2 molecules bind to IL-2 receptors on T helper cells and induce their proliferation.<sup>10</sup>

In patients with rheumatic fever and RHD, a depressed cellular immune response is now considered to have a central role in pathogenesis of rheumatic heart injury.<sup>11</sup> The immune system in rheumatic patients is challenged with an array of streptococcal antigens. The most important of these is blastogen A, which was found to have an effect on immunoglobulin synthesis and IL-1 and IL-2 formation.<sup>4</sup> In our study the significant increase of IL-2 (p<0.01, table 3) was found in patients with active RHD when compared with controls. This significant increase in IL-2 in active RHD can be explained on the basis of initiation of the cellular immune response by streptococcal blastogen A (antigen) ending in secretion of IL-2.<sup>10</sup>

In a previous study IL-2 was found to increase the cell mediated cytotoxicity,<sup>12</sup> while other investigators suggested the presence of cytotoxic cell species in patients with RHD that could be activated by streptococcal antigens leading to cellular damage during active rheumatic fever.<sup>13</sup> Those cells that have such cytolytic activity were found to be natural killer cells,<sup>14</sup> usually the activity of natural killer cells was found to be triggered by IL-2.<sup>15</sup> Data from these studies support the fact that the significant increase of IL-2 in the serum of our rheumatic patients means that IL-2 is important in the abnormal immune response in RHD via its effect on natural killer cell activity and the increase in cell mediated cytotoxicity.

Also one of the abnormal immune responses that has been reported in RHD is the increased natural killer cell cytotoxicity.<sup>2</sup> This observation can be explained by our findings of increased IL-2 production in rheumatic patients (p<0.01, table 3) as IL-2 is a lymphokine secreted from activated T lymphocytes and is responsible for the activity of natural killer cells, especially the cytotoxic character.<sup>15</sup> Thus we can suggest a sequence of events that is responsible for cellular damage in RHD: first stimulation of T lymphocytes by streptococcal antigens, this leads to secretion of IL-2, which results in increased natural killer cell cytotoxicity and the pathological changes in rheumatic patients.

In this study the Biotest Diagnostic Series of Clonab monoclonal antibodies (fluoro-chrome conjugated reagents for T cell typing) were used to determine T cell subsets in the studied groups of patients and controls. The CD3+, CD4+, and CD8+ monoclonal antibodies identify

respectively all mature peripheral blood T lymphocytes, helper T cells, and suppressor T cells.

The present study revealed that there was a significant decrease in CD8+ and CD3+ and significant increase in the H:S ratio in children with active RHD as compared with controls ( $p < 0.001$ , 0.01, 0.001, respectively, table 2). CD4+ cells showed a non-significant increase in the active group when compared with the controls (table 2).

Other studies done on patients with acute rheumatic fever revealed significant depression of OKT1+ and OKT4+ with insignificant increase in OKT8+ cells.<sup>16</sup> Another in vitro study on tonsillar tissues from rheumatic patients revealed an increased CD4+:CD8+ ratio after exposure to streptococcal products.<sup>17</sup>

Our children with non-active RHD showed a significant decrease in CD3+ only (table 2,  $p < 0.001$ ), with no significant change in IL-2, CD4+, CD8+, and the H:S ratio. These results imply that deficient CD3+ can be considered a constant finding in RHD whether active or not.

By comparing active and non-active RHD we observed a significant increase in IL-2 and the H:S ratio with a significant decrease in CD8+ cells. Thus a constant increase of IL-2 and a decrease in CD8+ cells were observed in patients with active RHD in comparison with both controls (tables 2 and 3, respectively) and the non-active RHD group. These data may add another constant finding that is related to rheumatic activity.

#### CONCLUSION

Deficient CD3+ cells can be considered a constant finding in patients with RHD, whether active or non-active. Also the significant increase in serum concentrations of IL-2 and the significant decrease in CD8+ cells can be considered

as a finding related to rheumatic activity. Finally another study has been planned to evaluate tumour necrosis factor- $\alpha$ , interleukin-I B (IL-IB), and natural killer cell function in active and non-active RHD.

- 1 Gillis S, Ferm MM, Ou W, Smith KA. T cell growth factor: parameters of production and quantitative microassay for activity. *Journal of Immunological Reviews* 1978;120:207-32.
- 2 Smith KA. T cell growth factor. *Journal of Immunological Reviews* 1980;51:337-41.
- 3 Zabriskie JB. The relationship of streptococcal cross-reactive antigens to rheumatic fever. *Transplant Proc* 1969;1:968-75.
- 4 Gray ED, Abdin Z, El-Kholy A, et al. Augmentation of cytotoxic activity by mitogens in rheumatic heart disease. *J Rheumatol* 1988;15:1672-6.
- 5 Committee on Rheumatic Fever and Bacterial Endocarditis of the American Heart Association. *Jones criteria (revised) for guidance in the diagnosis of rheumatic fever*. Dallas: American Heart Association, 1982.
- 6 Buffene GJ. Principle of immunochemical technique. In: Tietz NW, ed. *Fundamentals of clinical chemistry*. London: W B Saunders, 1987:144-59.
- 7 Boyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scan J Clin Lab Invest* 1967;21(suppl 97):77-89.
- 8 Scheijnius A, Fischer T, Forsum U, et al. Phenotypical characterization in situ of inflammatory cells in allergic and irritant contact dermatitis in man. *Clin Exp Immunol* 1984;55:81.
- 9 Morrison D. *Multivariate statistical methods*. New York: McGraw-Hill, 1976:134-8.
- 10 Daniele RP. *Immunology and immunologic diseases of the lung*. Oxford: Blackwell Scientific Publications, 1989:503-9.
- 11 Leuker RD, Wilhams RC Jr. Decreased reactivity of lymphocytes in mixed leucocyte culture from patients with rheumatic fever. *Circulation* 1972;46:655-60.
- 12 Svedersky LP, Shepart HM, Spencer SA, et al. Augmentation of human natural cell-mediated cytotoxicity by recombinant human interleukin-2. *J Immunol* 1984;133:714-8.
- 13 Yang LV, Soprey PR, Wittner MK, et al. Cell mediated immune destruction of cardiac myofibers in vitro. In: Read SE, Zabriskie JB, eds. *Streptococcal diseases and the immune response*. New York: Academic Press, 1980:633-47.
- 14 Lanier IL, Phillips JH. Evidence for three types of human cytotoxic lymphocyte. *Immunol Today* 1986;7:132-4.
- 15 Klein J. Lymphokines and their receptors. In: Klein J, ed. *Immunology*. Oxford: Blackwell Scientific Publications, 1990:227-45.
- 16 Bhatnagar PK, Nijhawan R, Prakash K. T cell subsets in acute rheumatic fever, rheumatic heart disease and acute glomerulonephritis cases. *Immunol Lett* 1987;15:217-9.
- 17 Regelman WE, Gray ED, Wannamaker LW, et al. Lymphocyte subpopulations in rheumatic heart disease. *J Rheumatol* 1987;14:23-7.