

The *in vivo* effect of triethylphosphine gold (auranofin), sodium aurothiomalate and azathioprine on lymphocyte subsets of patients with rheumatoid arthritis

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The percentages of CD5+ (pan T), CD8+ (mainly T suppressor) and CD4+ (mainly T helper) cells were studied in 80 patients with rheumatoid arthritis (RA), grouped according to medication into four groups: 1) controls, not receiving remission-inducing therapy, 2) patients on oral gold (triethylphosphine gold, auranofin), 3) patients on parenteral gold (sodium aurothiomalate) and 4) patients on azathioprine. The total number of lymphocytes was not influenced by treatment with auranofin or sodium aurothiomalate, while the number of lymphocytes was depressed in the azathioprine-treated group ($P < 0.01$). Neither treatment with azathioprine or parenteral gold, nor treatment with azathioprine influenced the CD4+/CD8+ ratio.

Key words: auranofin; sodium aurothiomalate; azathioprine; rheumatoid arthritis; lymphocyte subsets.

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A wide range of immunological processes have been implicated in the chronic synovitis of rheumatoid arthritis (RA). Large numbers of T cells are present in the synovium; through release of various lymphokines these cells contribute to the inflammation of RA and its regulation. Gold compounds and azathioprine can induce suppression of disease activity in patients with RA. However, the mechanisms whereby the administration of these agents suppress synovial inflammation in RA patients remain to be fully explained. The aim of the present paper was to examine if treatment of RA patients with triethylphosphine gold (auranofin), sodium aurothiomalate or azathioprine influenced the lymphocyte subpopulations expressing the CD4+ and CD8+ cell phenotypes.

PATIENTS AND METHODS

Patients

Eighty patients with classical or definite RA according to the ARA criteria were studied. They were divided into four groups according to medication: 1) controls, who had not received remission-inducing therapy for at least half a year, and who had never received treatment with immunosuppressive drugs ($n = 20$), 2) patients on oral gold (auranofin) 6 mg/day for more than 5 months ($n = 22$), 3) patients on parenteral gold (sodium aurothiomalate) 50 mg weekly to 50 mg every 2nd month, minimal total dose 500 mg ($n = 18$), and 4) patients on azathioprine 100-150 mg/day for more than 6 months ($n = 20$). Twenty-two patients received

treatment with glucocorticosteroids 2.5 to 7.5 mg/day. Median age was 58.4 years (range 16–79), and the groups were comparable with regard to age and sex.

The ratio of seropositive to seronegative patients was the same in the three groups receiving remission-inducing therapy, while there were more seronegative donors in the control group. There was no significant difference in erythrocyte sedimentation rate (ESR) and plasma orosomucoid concentration among the groups.

Isolation of cells

Mononuclear cells were isolated from heparinized blood immediately after blood sampling by Ficoll gradient centrifugation. The cells were washed three times with Hanks' buffered salt solution (HBSS). Mononuclear cells were frozen (1°C/min, controlled gradient freezing machine Cryoson, GB) in RPMI with 20% FCS and 10% dimethylsulphoxide and stored in liquid nitrogen until use. The percentage of dead cells after freezing and thawing was less than 5%.

Cell surface marker analysis by flow cytometry

The following monoclonal antibodies were used: Leu1 (anti-CD5), Leu2 (anti-CD8) and Leu3 (anti-CD4) (Becton-Dickinson). 1×10^6 blood mononuclear cells were washed twice in PBS with 5% FCS, resuspended in 100 μ l PBS containing FCS and 5 μ l of the monoclonal

antibody. After incubating the cells on ice for 45 min, the cells were washed twice in Isoton III (balanced electrolyte solution) and finally resuspended in Isoton III. Labelled cells were then analysed by flow cytometry using a fluorescence-activated cell sorter analyser (FACS, Becton-Dickinson), 5000 cells were counted.

Clinical chemical tests. Standard laboratory procedures were employed for estimation of lymphocyte concentration.

Statistical differences

The significance of the observed differences was calculated by the Mann-Whitney test, $P < 0.05$ was considered significant.

RESULTS

The lymphocyte count, the percentages of CD5+, CD8+ and CD4+ cells and the CD4/CD8 ratio in the four groups of patients are shown in Table 1. The concentration of lymphocytes was significantly lower in the azathioprine group ($P < 0.01$), while it was normal in the two groups treated with gold. Furthermore, the concentration of CD4+ and CD8+ cells was suppressed in the azathioprine group. There was no significant difference between the percentages of CD5+ and CD8+ cells in the four groups, while there was a significantly higher proportion of CD4+ cells in the azathio-

Table 1
CD5+, CD8+ and CD4+ cells (medians, ranges)

Treatment	Lymphocyte count ($10^9/l$)	CD5 %	CD8 %	CD4 %	CD4/CD8
Controls	2.1 (1.2–2.4)	62.5 (37–72)	17.0 (8–30)	45.0 (30–62)	2.3 (1.1–6.7)
Oral gold*	1.8 (1.2–3.3)	60.0 (43–72)	14.5 (4–39)	44.5 (32–64)	3.4 (1.0–13.9)
Parenteral gold**	1.9 (1.4–3.9)	60.0 (50–70)	17.0 (12–34)	42.0 (26–52)	2.8 (1.0–4.6)
Azathioprine	1.4+ (0.7–1.9)	67.0 (59–80)	22.5 (6–35)	51.0++ (33–65)	2.3 (0.9–9.3)

*auranofin, **sodium aurothiomalate

+ significantly lower than controls ($P < 0.01$)

++ significantly higher than controls ($P < 0.02$)

prine group ($P < 0.02$). The reason why the sum of the CD4+ and CD8+ cells exceeds the CD5+ cells in the azathioprine group is not known. There was, however, no significant difference between the ratio CD4/CD8 in the four groups.

DISCUSSION

Gold compounds and azathioprine influence cell-mediated immunity. Auranofin and sodium aurothiomalate were capable of stimulating compromised oxazolone-induced contact sensitivity, but neither agent significantly altered the uncompromised response in normal mice and rats (5). A suppressed skin test response to dinitrochlorobenzene (DNCB) was observed in patients receiving auranofin (2) and azathioprine (7) versus a normal response in patients receiving sodium aurothiomalate (2).

In phytohaemagglutinin (PHA)-stimulated human lymphocytes, auranofin but not sodium aurothiomalate inhibited the incorporation of 3-H-thymidine into DNA (1). Lymphocytes from patients with RA who were treated with auranofin showed suppression of PHA-stimulated incorporation of 3-H-thymidine into DNA (3), while the PHA response of lymphocytes from RA patients on azathioprine was normal (6).

To our knowledge it has not previously been described whether treatment of RA patients with oral or parenteral gold influence the T helper and T suppressor cells, while it has been shown that immunosuppression of renal allograft recipients with azathioprine and prednisolone do not influence T helper/T suppressor ratio (4). In accordance with the latter we find that treatment of RA patients with azathioprine reduces the total number of lymphocytes without altering the ratio of CD4+/CD8+ cells.

Further, we find that treatment of RA patients with oral or parenteral gold neither influences the lymphocyte concentration, nor the CD4/CD8 ratio.

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REFERENCES

1. Finkelstein, A. E., Burrone, O. R., Walz, D. T. & Misher, A.: Effect of auranofin on DNA and protein synthesis in human lymphocytes. *J. Rheumatol.* 4, 245-251, 1977.
2. Lorber, A., Jackson, W. H. & Simon, T. M.: Assessment of immune response during chrysotherapy. Comparison of gold sodium thiomalate vs. auranofin. *Scand. J. Rheumatol.* 10, 129-137, 1981.
3. Lorber, A., Kunishima, D. H., Harralson, A. F. & Simon, T. H.: Unbound versus total serum concentration: pharmacological actions on cellular function. *J. Rheumatol.* 10, 568-573, 1983.
4. Sweny, P. & Tidman, N.: The effect of cyclosporin A on peripheral blood T cell subpopulations in renal allografts. *Clin. Exp. Immunol.* 47, 445-448, 1982.
5. Walz, D. T. & Griswold, D. E.: Immunopharmacology of gold sodium thiomalate and auranofin. Effects on cell-mediated immunity. *Inflammation* 3, 117-128, 1978.
6. Yu, D. T., Clements, P. J. & Peter, J. B.: Lymphocyte characteristics in rheumatic patients and the effects of azathioprine therapy. *Arthritis Rheum.* 17, 37-45, 1974.
7. Zweiman, B.: Immunosuppressive effects of specific classes of agents with special reference to organ transplantation: immunosuppression by thiopurines. *Transplant. Proc.* 5, 1197-1201, 1973.

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