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Effect of colchicine on T cell subsets of healthy volunteers

D. ILFELD*, E. FEIERMAN†, O. KUPERMAN‡, S. KIVITY†, M. TOPILSKY†, L. NETZER§, M. PECHT§ & N. TRAININ§ **Department of Medicine 'C', Beilinson Medical Center, Petah Tikva, †Institute of Pulmonary and Allergic Diseases, Ichilov Medical Center, Tel Aviv,‡ Clinical Immunology Laboratory, Soroka Medical Center, Beer Sheva & §Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel*

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Summary. We examined the effect of oral colchicine (1–2 mg/day) on four healthy volunteers' T cell subsets. Colchicine significantly ($P < 0{\cdot}01$) decreased the mean ($\pm$SD) percent of OKT3$^+$ total T cells (from 70 ± 16 to 47 ± 13), OKT4$^+$ helper/inducer T cells (from 44 ± 9 to 24 ± 6), and OKT8$^+$ suppressor/cytotoxic T cells (from 27 ± 7 to 17 ± 7), but did not significantly affect the OKT4:OKT8 ratio (from $1{\cdot}64 \pm 0{\cdot}21$ to $1{\cdot}48 \pm 0{\cdot}45$) or concanavalin A-induced suppressor cell function (from $44 \pm 9\%$ to $47 \pm 13\%$). Thus, colchicine non-selectively decreased the circulating helper/inducer and suppressor/cytotoxic T cells.

Oral colchicine treatment (1–2 mg/day) corrected the deficiency of concanavalin A (Con A)-induced suppressor cell function and elevated OKT4$^+$ helper/inducer to OKT8$^+$ suppressor/cytotoxic T cell ratio in patients with familial Mediterranean fever (FMF) (Ilfeld & Kuperman, 1982; Melamed *et al.*, 1983; Schlesinger *et al.*, 1983). *In vitro* colchicine at 10^{-5} M also corrected FMF patients' deficiency of Con A-induced suppressor cell function (Ilfeld & Kuperman, 1982; Schlesinger *et al.*, 1983). These studies showed that oral colchicine directly acted on FMF patients' T lymphocytes and therefore suggested that colchicine may be an immunomodulating drug. In the present paper, we studied the effect of oral colchicine on healthy volunteers' Con A-induced suppressor cell function and proportions of OKT3$^+$ total T cells, OKT4$^+$ helper/inducer T cells, and OKT8$^+$ suppressor/cytotoxic T cells in order to determine whether oral colchicine can influence T lymphocytes when there is no immunoregulatory abnormality.

Correspondence: Dr David Ilfeld, Department of Microbiology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69 978, Israel.

Healthy volunteers aged 23–39 years were studied. The study was approved by Beilinson Medical Center's Helsinki Committee and the four healthy volunteers gave written informed consent before receiving oral colchicine. Blood was drawn between 0800 and 1000 hours (2–4 hr after the last dose of colchicine) and peripheral blood mononuclear cells (PBMC) were isolated on Ficoll-isopaque gradients. To measure Con A-induced suppressor cell function, 5×10^6 PBMC were preincubated for 44 hr in 1 ml of RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS), with or without 10 μg/ml of Con A (Miles Yeda), and with or without colchicine (Sigma). These suppressor cells were incubated for 30 min with 50 μg/ml of mitomycin C, washed three times, and 10^5 of these cells were cocultured for 72 hr with 10^5 fresh PBMC from allogeneic healthy volunteers and 1 μg/ml of purified phytohaemagglutinin PHA (Wellcome) in 0·2 ml of RPMI 1640 (including 10% FCS). Percent suppression represents the percent difference between the net counts per minute (c.p.m.) of tritiated thymidine uptake of PHA-stimulated responder cells cocultured with suppressor cells preincubated with Con A and/or colchicine and the net CPM of PHA-stimu-

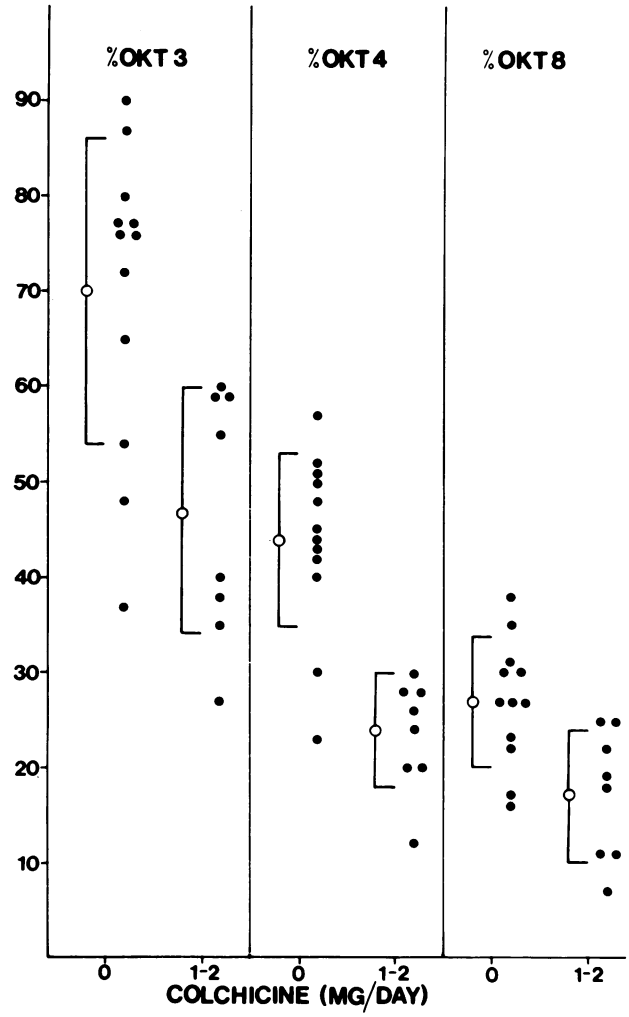

Figure 1. Effect of oral colchicine (1–2 mg/day) on the percent of $OKT3^+$, $OKT4^+$, and $OKT8^+$ T lymphocytes in four healthy volunteers. The individual results are shown by closed circles, the mean by open circles, and the standard deviation by bars for the untreated and colchicine-treated healthy volunteers.

lated responder cells cocultured with suppressor cells preincubated in medium.

For measuring T cell subsets, 10^6 PBMC were suspended in RPMI 1640 with 5% FCS, mixed with monoclonal antibodies OKT3, OKT4, and OKT8 (Ortho) at a final dilution of 1:40 in an ice-water bath for 30 min, washed twice, mixed with fluorescein conjugated rabbit anti-mouse IgG at a final dilution of 1:40 in an ice-water bath for 30 min, washed, and measured on a fluorescence activated cell sorter (FACS II, Beckton & Dickinson). Statistical significance was calculated by the two-tailed Student's *t*-test.

Four healthy male volunteers were treated with oral colchicine for 2 weeks. Two received 0·5 mg twice daily, one received 1·5 mg/day, and the other one received 1 mg twice daily. They were tested for Con A-induced suppressor cell function, proliferation to mitogens, and percent of $OKT3^+$, $OKT4^+$, and $OKT8^+$ cells at least once before treatment, twice after 6–13 days of oral colchicine treatment, and at least once after stopping colchicine treatment more than 4 weeks previously. There was a significant difference

between these four untreated and colchicine-treated healthy volunteers' mean (±SD) percent of $OKT3^+$ cells (70±16 and 47±13, respectively, $P<0.005$), percent of $OKT4^+$ cells (44±9 and 24±6, respectively, $P<0.001$), and percent of $OKT8^+$ cells (27±7 and 17±7, respectively, $P<0.01$) (Fig. 1). The four healthy volunteers' absolute lymphocyte counts were decreased during oral colchicine treatment (1972±487 per mm^3) as compared to without treatment (2451±533 per mm^3) which shows that the absolute number of $OKT3^+$, $OKT4^+$, and $OKT8^+$ cells was decreased by oral colchicine. The four colchicine-treated healthy volunteers' percent of $OKT3^+$ cells, $OKT4^+$ cells, and $OKT8^+$ cells were also significantly decreased as compared to untreated control healthy volunteers tested in the same assays as the colchicine-treated healthy volunteers (data not shown). There was no significant difference in the OKT4:OKT8 ratio without treatment (1·64±0·21) and during oral colchicine treatment (1·48±0·45). Similarly, there was no significant difference of Con A-induced suppressor cell function without treatment (44±9%) and during oral colchicine treatment (47±13%). In contrast, a pharmacological concentration of *in vitro* colchicine (10^{-8} M) significantly ($P<0.001$) decreased these four healthy volunteers' (when they were not treated with oral colchicine), as well as 10 other healthy volunteers' Con A-induced suppressor cell function from 41±19% to 13±12%. Incubation of PBMC with 10^{-8} M colchicine (without Con A) for 44 hr had no significant effect when these cells were cocultured with PHA and fresh allogeneic responder cells.

There was a mild but not significant difference between the four untreated and colchicine-treated healthy volunteers' proliferation to PHA (161,000±50,000 and 123,000±84,000 c.p.m., respectively) and to Con A (134,000±79,000 and 100,000±37,000 c.p.m., respectively). Similarly, a pharmacological concentration (10^{-8} M) of *in vitro* colchicine decreased proliferation to PHA by 31%.

The different effects of *in vitro* and *in vivo* colchicine on Con A-induced suppressor cell function suggest the existence of different mechanisms of action. For example, *in vivo* colchicine may cause a redistribution of T cells from the peripheral blood to extravascular sites such as the spleen, lymph nodes, thoracic duct, and bone marrow as occurs with glucocorticosteroid treatment (Fauci & Dale, 1975). This hypothesis of colchicine causing a redistribution between intravascular and extravascular compartments could be due to alterations in T lymphocytes' surface membrane, since alteration of molecular configurations on the surface of lymphocytes markedly changes their circulation patterns (Gesner & Ginsburg, 1964; Woodruff & Gesner, 1968). Alternatively, the colchicine-induced reduction of circulating T lymphocytes in healthy volunteers may represent an adverse drug reaction such as a toxic side effect. It does not appear to be an idiosyncratic reaction or an allergic reaction because these are unusual reactions and thus unlikely to be observed in all four volunteers.

Colchicine injected, together with antigens, into mice increased the circulating antigen-specific antibody titre via the inactivation or elimination of suppressor cells or their precursors (Shek & Coons, 1978; Shek, Waltenbaugh & Coons, 1978). These results may be due to colchicine's anti-mitotic effect of disrupting microtubular assembly, since vinblastine (which also blocks microtubules) had similar effects while lumicolchicine (which does not block microtubules) was ineffective. The pharmacological doses of oral colchicine received by our healthy volunteers mildly decreased the *in vitro* proliferation of PBMC to PHA and Con A. Furthermore, the T cells removed from the pool of circulating T cells may have been more sensitive to the anti-mitotic activity of colchicine. Therefore, the non-selective reduction of the healthy volunteers' circulating helper/inducer and suppressor/cytotoxic T lymphocytes may be due to an anti-mitotic effect. Colchicine has other cellular effects such as modulating prostaglandin E and cyclic AMP, so further research is needed to determine the mechanism of action of colchicine's effect on healthy volunteers' T cells.

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