Clin. exp. Immunol. (1989) **78**, 230–232

Inhibition of delayed hypersensitivity reactions by colchicine. II. Colchicine inhibits interferon-gamma induced expression of HLA-DR on gut epithelial cell line

Y. A. MEKORI*†, Y. CHOWERS†, I. DRUCKER†, A. KLAJMAN†‡ **Allergy–Immunology Unit; and † Laboratory for Clinical Immunology, Meir Hospital, Kfar Saba; and ‡ The Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel*

(Accepted for publication 7 July 1989)

SUMMARY

The effects of colchicine and vinblastine on interferon-gamma (IFN-γ) induced expression of HLA-DR antigens on the HT-29 colonic carcinoma cell line was investigated *in vitro*. Both drugs prevented the expression of HLA-DR only if applied before or together with IFN-γ. Methotrexate, an anti-mitotic drug, failed to inhibit DR expression by these cells. Colchicine and vinblastine, but not methotrexate, act on the cellular microtubules, interfering with the transport of proteins from the protoplasm to the cell surface and thus preventing the appearance of HLA-DR antigens. The reported findings may explain the role of colchicine in preventing delayed hypersensitivity reactions.

Keywords colchicine interferon-gamma HLA-DR antigen

INTRODUCTION

Colchicine is a unique anti-inflammatory agent whose traditional use has been in the treatment and prophylaxis of acute gout (Malkinson, 1982). Colchicine is also effective against a variety of other diseases (Malkinson, 1982). The mechanism of its beneficial effect in these diseases is not clear. Colchicine binds to tubulin, blocks mitosis and inhibits a variety of functions of polymorphonuclear leucocytes both *in vivo* (Ehrenfeld *et al.*, 1980) and *in vitro* (Rudolph, Greengard & Malawista, 1977). Traditionally, the anti-inflammatory effects of colchicine were attributed to its anti-mitotic properties and to impairment of granulocyte chemotaxis.

Recently, the inhibitory effects of colchicine on cell-mediated immune responses have been noticed. Lyons *et al.* (1986) found that colchicine inhibited the clinical and histopathological manifestations of experimental allergic encephalitis and the delayed hypersensitivity response (DHR) to myelin basic protein. More recently we have shown that colchicine inhibited the elicitation but not the induction of contact sensitivity reactions in mice (Mekori *et al.*, 1989). By using adoptive transfer experiments we could demonstrate that the drug interfered with primary T cell effector function but not with T helper cell priming (Mekori *et al.*, 1989). We suggested that one of the factors to be considered in analysis of the inhibitory influence of colchicine on DHR is its effect on the accessory cells that mediate T cell activation *in vivo*.

To characterize further the inhibition of DHR by colchicine, we analysed in the present study its effect on interferon-gamma (IFN-γ) induced HLA-DR expression by the HT-29 colonic carcinoma cell line. We showed that colchicine in concentrations of 10^{-8} M and 10^{-7} M profoundly inhibited the IFN-γ-induced expression of the HLA-DR antigen on the HT-29 cells. However, another anti-mitotic drug (methotrexate) did not alter class II antigen expression on these cells.

Correspondence: A. Klajman, M.D., Laboratory for Clinical Immunology, Meir Hospital, 44281 Kfar Saba, Israel.

MATERIALS AND METHODS

The effects of colchicine were examined on IFN-γ- induced HLA-DR expression by the HT-29 cell line derived from human colonic carcinoma (kindly provided by Dr Y. Markuson, Cancer Research Institute, Hadassah Medical School, Jerusalem).

Cell culture technique

The method described recently by Mayer and Shlien (1987) was adopted. HT-29 cells were grown as an adherent monolayer in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 4·5 g glucose/l, antibiotics, and 2 mM glutamine (culture medium). Cells were grown in 50-ml flasks (Nunclon Delta, Nunc, Roskilde, Denmark) at 37°C in a humidified atmosphere of 5% CO_2 in air.

Evaluation of HLA-DR expression

The HT-29 cells were trypsinized with trypsin-EDTA solution (Beth Haemek, Israel) and recultured on glass cover-slips placed in Four Wells Multidish (Nunc), 10^5 cells in 1 ml culture medium per well. They were incubated in 5% CO_2 at 37°C. After 24 h the cells had re-adhered and the wells were washed to remove dead

Table 1. Effect of colchicine on interferon-gamma (IFN-γ) induced expression of HLA-DR

	Fluorescent cells (%)		
Colchicine concentration	IFN-γ post colchicine*	Washing†	IFN-γ prior to colchicine‡
—			86±6(4)
10^{-8} M	52(1)	96±2·8(2)	ND
10^{-7} M	2·8±3·1(4)	48±19(4)	86±9·8(2)

The number of experiments performed is shown in parentheses.

Results are expressed as mean±s.d.

* Cells incubated first with colchicine for 24 h then exposed to IFN-γ for 48 h.

† Cells incubated first with colchicine for 24 h, washed and then exposed to IFN-γ for 48 h.

‡ Cells exposed first to IFN-γ for 48 h and then to colchicine for 24 h.

ND, not done.

cells. For the evaluation of HLA-DR expression fresh culture medium alone, or culture medium containing human recombinant IFN-γ (1000 U/ml, a gift of Inter-Yeda, Nes Ziona, Israel) was layered on top for 48 h. Subsequently the cover-slips with the cells were washed and stained for the presence of HLA-DR by indirect immunofluorescence using monoclonal mouse anti-human HLA-DR antibody (Dako, Glostrup, Denmark) and counterstained with fluorescein-conjugated rabbit anti-mouse IgG (Dako). After washing and thorough drying, the cells were fixed in 5% acetic acid in absolute alcohol for 10 min at −20°C, washed, and the fluorescent cells were counted under a Zeiss u.v. microscope with epicondenser.

Effect of colchicine, vinblastine and methotrexate on the induction of HLA-DR antigens by IFN-γ

Colchicine (for i.v./i.m. injection dissolved in 0·95% saline, E. Lilly & Co., Indianapolis, IN) was added to the wells in concentrations of 10^{-7} M or 10^{-8} M, 24 h before, together with IFN-γ, or after 48 h incubation with IFN-γ. Half of the wells which had been pre-incubated with colchicine were washed prior to the addition of IFN-γ, and the second half remained unwashed (Table 1).

Cells were incubated with vinblastine (Teva Pharmaceutical Industries, Petach Tikva, Israel) in concentrations of 10^{8}–10^{-5} M for 6 h before being incubated with IFN-γ. Vinblastine was dissolved in 0·95% saline, pH adjusted to 7·2 with NaOH. Half of the wells were washed and half remained unwashed before IFN-γ was added for 48 h. Methotrexate in concentrations of 10^{-5}–10^{-8} M was added for 24 h before, or for 48 h together with IFN-γ. The required dilutions of the drugs were made with DMEM.

RESULTS

HT-29 cells cultured in medium alone were negative for HLA-DR. In contrast, a high percentage of cells cultured in the presence of IFN-γ for 48 h were strongly positive for DR (>90%). As seen in Table 1, this effect could be inhibited by the addition of colchicine prior to incubation with IFN-γ.

Table 2. Effect of vinblastine on interferon-gamma (IFN-γ) induced expression of HLA-DR

	Fluorescent cells (%)		
Vinblastine concentration (M)	IFN-γ post vinblastine*	Washing†	IFN-γ prior to vinblastine‡
10^{-8}	74	100	ND
10^{-7}	60	100	91
10^{-6}	48	78	90
10^{-5}	22	ND	79

* Cells incubated first with vinblastine for 24 h and then exposed to IFN-γ for 48 h.

† Cells incubated first with vinblastine for 24 h, washed and then exposed to IFN-γ for 48 h.

‡ Cells exposed first to IFN-γ for 48 h and then to vinblastine for 24 h.

One set of experiments was performed.

Pretreatment of the cells with colchicine for 24 h and subsequent incubation with IFN-γ for 48 h decreased the number of positive HLA-DR cells to 3% (colchicine 10^{-7} M) and to 52% (colchicine 10^{-8} M). When the cells were washed at the end of a 24-h incubation with colchicine the effect was significantly weaker, the number of positive cells being 44% (10^{-7} M) and 98% (10^{-8} M). Addition of colchicine for 24 h after the 48-h incubation with IFN-γ had little if any effect on the expression of HLA-DR antigen. Concomitant incubation of the cells with both colchicine and IFN-γ abrogated the appearance of HLA-DR (data not shown).

The effect of vinblastine, another anti-tubulin drug (Zakhireh & Malech, 1980), on HLA-DR expression by HT-29 cells was similar to that of colchicine (Table 2), although colchicine was somewhat more potent on a molar basis. The action of colchicine and vinblastine was not due to their toxic effect on the target cells as the viability of these cells, by trypan blue exclusion, was always >95% after exposure to these drugs.

The folic acid agonist methotrexate, however, which is a potent anti-mitotic drug, failed to effect HLA-DR expression at the concentration range of 10^{-8}–10^{-6} M (data not shown).

DISCUSSION

To characterize further the inhibition of DHR by colchicine we analysed its effects on HLA-DR antigen expression. Expression of the class II major histocompatibility complex (MHC) antigen is required for antigen presentation and induction of immune responses (Rosental & Shevach, 1977; Weinberger *et al.*, 1980). Several non-lymphoid tissues have been found to express HLA-DR antigens normally or under special circumstances. These include cutaneous Langerhans cells, keratinocytes, epithelium of the gastrointestinal tract, Kupffer cells, endothelial cells, etc. (Natali *et al.*, 1981; Basham *et al.*, 1984). IFN-γ has been found to be a major inducer of HLA-DR expression by both lymphoid and non-lymphoid tissues (Basham *et al.*, 1984; Manyak *et al.*, 1988).

Induction of class II MHC molecules on epithelial, endothelial and other cells by IFN-γ correlates with their ability to present antigen to T cells (Manyak *et al.*, 1988). In this context,

the inhibition of class II antigen induction could down-regulate immune responses. Indeed, Bland & Warren (1986) have demonstrated that the accessory cell function of gut epithelial cells was blocked by anti-Ia monoclonal antisera. Manyak *et al.* (1988) showed that IFN-α inhibited the IFN-γ-induced expression of class II MHC molecules by endothelial cells. This inhibition was pronounced when IFN-α was added before or simultaneously with IFN-γ.

It is interesting that colchicine and vinblastine, but not the potent anti-mitotic drug methotrexate, could prevent induction of HLA-DR by IFN-γ on HT-29 cells. The action of methotrexate differs markedly from that of the other two drugs. It interrupts the synthesis of DNA and RNA by producing an acute intracellular deficiency of folate coenzymes. To be effective the two drugs had to be applied before or together with IFN-γ. They were ineffective once HLA-DR antigens had already been expressed. The continuous presence of colchicine and vinblastine was necessary to establish their maximal effect. We believe that this action of colchicine and vinblastine was not due to their toxic effect on the target cells since the viability of these cells (by trypan blue exclusion) was always >95%. In addition, the fact that colchicine did not inhibit DR expression when added after pre-incubation with IFN-γ also argues against a cytotoxic effect of the drug. Furthermore, as commercially available colchicine and vinblastine were dissolved in 0·95% saline, this solvent could not have had any deleterious effect.

If *in vitro* findings are applicable *in vivo*, the implications may be highly significant. IFN-γ has been demonstrated at inflammatory sites (Geppert & Lipsky, 1985). This would suggest that T cells activated by viruses, bacteria or other factors can produce IFN-γ, leading to the induction of HLA-DR or Ia on cells that do not normally express them. The data presented by several authors (Geppert & Lipsky, 1985; Bland & Warren, 1986; Mayer & Shlien, 1987) suggest that these HLA-DR positive cells may function as antigen-presenting cells and so may participate in immunologically mediated inflammation and induce autoimmune reactions.

MHC class II antigen expression at the light microscope level in isolated enterocytes appears to be restricted to the basolateral enterocyte membrane (Bland & Warren, 1986). Although granular staining in the supranuclear region has been noted both in tissue sections and in isolated enterocytes, the ultrastructural localization of enterocyte class II molecules on specific organelles has not been reported (Bland, 1988). The appearance under light microscopy was consistent with intracellular accumulation within the endosome compartment in the apical cytoplasm of the enterocytes (Bland, 1988). Preliminary studies in our laboratory have also demonstrated that colchicine inhibits the intracellular expression of the HLA-DR (unpublished).

Examples of diverse phenomena induced by colchicine and considered to be modulated by microtubules include effects on cellular ultrastructure (Zakhireh & Malech, 1980), lysosome movement and endosome–lysosome fusion, the translocation of proteins from the rough endoplastic reticulum to the Golgi complex and the transport of proteins from the Golgi complex to the cell membrane. Some notable effects of colchicine on DNA synthesis and cell turnover have also been observed (reviewed by Malkinson, 1982). Any or all of these effects on the various intracellular processes may explain the interference by colchicine with expression of the DR antigen.

In recent work colchicine has been demonstrated to prevent the expression of DHR in mice (Mekori *et al.*, 1988). We provide here the first direct evidence that colchicine and vinblastine inhibit the IFN-γ-induced expression of HLA-DR antigens by a colon epithelial cell line. It is possible that colchicine has more than one effect on DHR and may influence other participants in the response. Nevertheless, the finding of a previously unrecognized action of the drug may explain its ability to block expression of cellular immunity.

REFERENCES

Basham, T.Y., Nickoloff, B.J., Merigan, T.C. & Morhenn, V.B. (1984) Recombinant gamma interferon induces HLA-DR expression on cultured human keratinocytes. *J. Invest. Dermatol.* **83,** 88.

Bland, P. (1988) MHC class II expression by the gut epithelium. *Immunol. Today,* **9,** 174.

Bland, P.W. & Warren, L.G. (1986) Antigen presentation by epithelial cells of the rat small intestine. I. Kinetics, antigen specificity and blocking by anti Ia antisera. *Immunology,* **58,** 1.

Ehrenfeld, M., Levy, M., Bar Eli, M., Gallily, R. & Eliakim, M. (1980) Effect of colchicine on polymorphonuclear leukocyte chemotaxis in human volunteers. *Br. J. Clin. Pharmacol.* **10,** 297.

Geppert, T.D. & Lipsky, P.E. (1985) Antigen presentation by interferon-γ-treated endothelial cells and fibroblasts. *J. Immunol.* **135,** 3750.

Lyons, M.J., Amador, R., Petito, C., Nagashima, K., Weinreb, H. & Zabriskie, J.B. (1986) Inhibition of acute experimental allergic encephalitis by colchicine. *J. exp. Med.* **164,** 1803.

Malkinson, F.D. (1982) Colchicine, new uses of an old drug. *Arch. Dermatol.* **118,** 453.

Mayer, L. & Shlien, R. (1987) Evidence for function of Ia molecules on gut epithelial cells in man. *J. exp. Med.* **166,** 1471.

Manyak, C.L., Tse, H., Fischer, P., Coker, L., Sigal, N.H. & Koo, G.C. (1988) Regulation of class II MHC molecules on human endothelial cells. *J. Immunol.* **140,** 3817.

Mekori, Y.A., Baram, D., Goldberg, A. & Klajman, A. (1989) Inhibition of delayed hypersensitivity in mice by colchicine. I. Mechanism of inhibition of contact sensitivity *in vivo*. *Cell. Immunol.* **120,** 330.

Natali, P.G., De Martino, C., Quaranta, V., Nicorta, M.R., Frezza, F. & Ferrone, S. (1981) Expression of Ia-like antigens in normal human nonlymphoid tissues. *Transplantation,* **31,** 75.

Rosenthal, A.S. & Shevach, E.M. (1973) Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. exp. Med.* **138,** 1194.

Rudolph, S.A., Greengard, P. & Malawista, S.E. (1977) Effects of colchicine on cyclic AMP levels in human leukocytes. *Proc. natl. Acad. Sci. USA,* **74,** 3404.

Weinberger, O., Herman, S.M., Mescher, M.F., Benacerraf, B. & Burakoff, S.J. (1980) Cellular interactions in the generation of cytotoxic T lymphocyte responses: role of Ia-positive splenic adherent cells in presentation of H-2 antigen. *Proc. natl. Acad. Sci. USA,* **77,** 6091.

Zakhireh, B. & Malech, H.L. (1980) The effect of colchicine and vinblastine on the chemotactic response of human monocytes. *J. Immunol.* **125,** 2143.