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# Comparative activity of CP-17,193 and five established immunosuppressives toward the antigens SRBC and $EL_4$

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## SUMMARY

A new immunosuppressant, 2-(4′-chlorophenyl)benzothiopyrano-(4,3-*c*)pyrazol-3-one, i.e. CP-17,193, was compared to several standard immunosuppressants with respect to its ability to depress immune responses to two antigens, $EL_4$ tumour cells and sheep erythrocytes (SRBC). The order of suppressive potency on a weight basis was methotrexate > CP-17,193 > cyclophosphamide > 6-mercaptopurine > azathioprine > oxisuran for both antigens. In general, it took more immunosuppressant to inhibit the response to SRBC than to $EL_4$. This resulted in a different therapeutic index for immunosuppression against each antigen. CP-17,193 and cyclophosphamide preferentially inhibited the humoral immune response and were the only agents demonstrating such selectivity. CP-17,193's favourable therapeutic index and its structural dissimilarity from the antimetabolite and alkylating immunosuppressants suggests that it may act through a novel mechanism.

## INTRODUCTION

In a search for novel immune suppressant agents we have discovered CP-17,193, i.e. 2-(4′-chlorophenyl)-benzothiopyrano(4,3-*c*)pyrazol-3-one (see Fig. 1 for structure), which displayed marked immunosuppressive activity in several animal model systems. This compound is of interest because of its potency and its apparent selectivity for the humoral immune response, and because its structure suggests it will be neither an alkylating agent nor an antimetabolite. Its selection from among other chemical congeners is described elsewhere (Lombardino & Otterness, 1981). The object of this paper is to detail some of the immunological properties of CP-17,193 and to compare it to other immunosuppressives.

## MATERIALS AND METHODS

*Immune response to $EL_4$ cells.* This procedure was used essentially as described by Otterness & Chang (1976).

*(a) Immunization and drug dosing.* $EL_4$ cells ($1 \times 10^8$) were injected intraperitoneally into groups of 10 BALB/c mice (Jackson Laboratories, Bar Harbor, Maine). All mice from a single group were dosed orally with either saline or drug once each day from 3 days prior to 9 days post-immunization. Dosing was omitted on Sundays, usually days +2 and +9. CP-17,193 was administered on days 0 to +9 only. Measurements of cellular and humoral immunity were made on day 10. The $EL_4$ cell line was maintained as previously described (Otterness & Chang, 1976).

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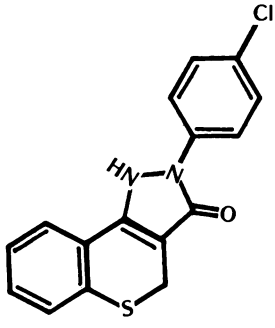


**Fig. 1.** Structure of CP-17,193.

*(b) Measurement of cell-mediated immunity.* Spleen cells were pooled from each group of BALB/c mice. After washing three times in Hanks' minimum essential medium (HMEM), the cells were resuspended in HMEM with 20% fetal calf serum to a concentration of $3 \times 10^7$ cells per ml. The spleen cell suspension (0·5 ml) was mixed with $5 \times 10^4$ $^{51}$Cr-labelled $EL_4$ cells (0·5 ml) in a 35-mm petri dish (Falcon Plastics, Oxnard, California) and incubated for 3 hr at 37°C in 10% $CO_2$ on a rocker platform making eight reciprocations per min. After incubation, the cells were resuspended, transferred into test-tubes with a 1-ml HMEM wash and centrifuged. The per cent specific lysis of $EL_4$ cells was calculated for each group of mice from the release of $^{51}$Cr into the supernatant after correction for the spontaneous release (2–3%) of label from $EL_4$ cells. All assays were performed in triplicate. The effect of drug treatment was computed as per cent inhibition of release, i.e. $100 \times (1 - \%$ release drug-treated/% release control). Although standard deviations are not shown in Figs 2–5, the replicate determinations generally differed by less than 3% within a given experiment. The validity of the data as to rank order and selectivity of immunosuppression was assured by independent repetition of the experiment.

*(c) Measurement of humoral immunity.* The sera from each group of 10 immunized BALB/c mice were collected, pooled and diluted in HMEM containing fetal calf serum (20%). A 0·2-ml sample, containing $2{\cdot}5 \times 10^5$ $^{51}$Cr-labelled $EL_4$ cells, was mixed with 0·3 ml of a 1/40 dilution of rabbit complement absorbed with $1 \times 10^8$ $EL_4$ cells/ml and then added to 0·5 ml of serial dilutions of the mouse sera. The dilution giving 50% lysis of $EL_4$ cells was determined from the van Krogh equation (Kabat & Meyer, 1961), and considered to be the titre of the serum. Drug effects were computed as the percentage decrease in the titre of sera when drug-treated mice were compared to saline-treated mice.

*The humoral immune response to sheep red blood cells (SRBC).* A modification of the Jerne plaque technique (Cunningham & Szenberg, 1968) was used to determine drug effects on the inductive phase of the humoral immune response. A 0·2-ml sample of packed SRBC was washed once in phosphate-buffered saline, pH 7·0, and diluted to a concentration of $2 \times 10^7$/ml. One millilitre of these cells was injected intraperitoneally into groups of six CD-1 mice (Charles River Breeding Laboratories, Wilmington, Massachusetts), 20–25 g, 6–8 weeks old. Drug-treated groups were dosed daily. On day 5, the mice were killed and a spleen cell suspension prepared in CMRL 1066. A 1·0-ml mixture was made at room temperature with 0·5 ml of a dilution of the spleen cells, 0·2 ml CMRL 1066, together with 0·1 ml of pooled, lyophilized guinea-pig complement and 0·2 ml SRBC ($2 \times 10^9$/ml). The final dilution of spleen cells was used such that 20–30 plaques per chamber were counted after a 60-min incubation at 37°C in 10% $CO_2$. The results are expressed as the number of plaque-forming cells per spleen.

*Cellular immune response to SRBC.* Cellular immunity in CD-1 mice was measured according to the procedure of Miller, Mackaness & Lagrange (1973). SRBC were washed once in phosphate-buffered saline and diluted to a concentration of $1 \times 10^8$/ml. Each mouse was injected subcutaneously with 0·1 ml of this suspension on day 0. Drug administration was commenced on day 0 and continued up to and including day 5. Five mice were examined at each dosage level. On day 5, $1 \times 10^8$ SRBC in 0·01 ml were injected into the subplantar region of the right foot. Twenty-four

hours later the right and left feet were sectioned at the tibiotarsal joint and weighed; the increase in weight of the injected foot was computed. The increase in weight due to immunization was then computed as the increase in weight of the immunized and challenged group minus the increase in weight of the control unsensitized but challenged group. The effect of drug treatment was computed as the percentage decrease in the response compared to the untreated group.

*Haemagglutination assay and therapeutic index measurement.* Groups of five CD-1 mice were immunized on day 0 with $1 \times 10^8$ SRBC intraperitoneally. Drug administration was carried out daily from day 0 to 10. On day 10, the mice were individually bled from the orbital plexus. The mice were observed until day 15 at which time all deaths were recorded. Twenty-five microlitres of the sera from each mouse were serially diluted in microtitre plates. To each well was added 50 $\mu$l of a 0·5% SRBC suspension in 100-fold-diluted fetal calf serum previously absorbed with packed SRBC and the plates were incubated for 4 hr at room temperature. The highest dilution of sera to give visible agglutination was scored as the titre of haemagglutinating antibody. An individual mouse was classed as immunosuppressed if its titre was 2 dilutions below the titre of the individual control mice.

*Drugs.* 6-Mercaptopurine was purchased from Nutritional Biochemicals, Cleveland, Ohio. Cyclophosphamide, azathioprine and methotrexate were obtained as gifts from Mead–Johnson, Evansville, Illinois, Burroughs–Wellcome, Research Triangle Park, North Carolina, and Lederle Laboratories Division, American Cyanamid, Pearl River, New York, respectively. Oxisuran (Freedman *et al.*, 1972) and CP-17,193 (Lombardino & Otterness, 1981) were prepared for these studies by Dr J. G. Lombardino of these laboratories.

## RESULTS

*Relative potency of suppression of the cellular response to $EL_4$ cells.* CP-17,193 was compared with five immunosuppressants—azathioprine, cyclophosphamide, 6-mercaptopurine, methotrexate and oxisuran—for its ability to suppress the cellular immune response to $EL_4$ tumour cells (Fig. 2). On a mg/kg basis, CP-17,193 was more potent than cyclophosphamide, azathioprine and 6-mercaptopurine, giving 50% inhibition of the immune response at 1·6 mg/kg, p.o. Cyclophosphamide, azathioprine and 6-mercaptopurine gave 50% inhibition at 8, 10 and 25 mg/kg p.o.

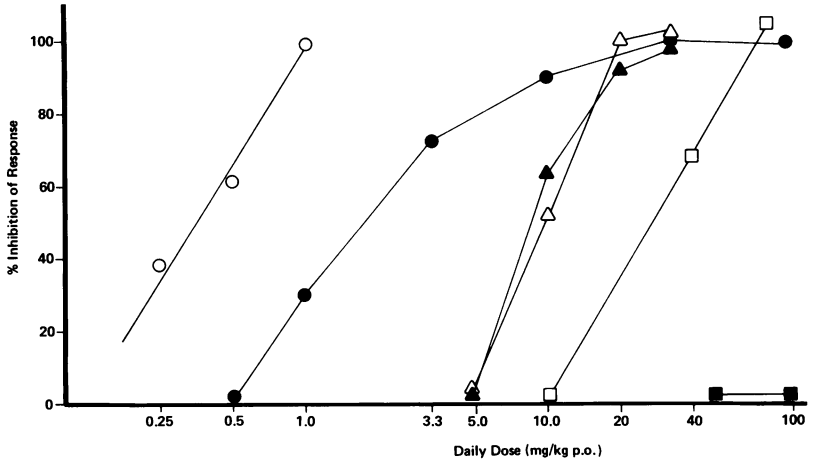


**Fig. 2.** The effect of immunosuppressive drugs on the cellular immune response to $EL_4$ cells. Groups of 10 mice were immunized on day 0 with $1 \times 10^8$ $EL_4$ cells and the response measured on day 10 as described under Materials and Methods. Each group was treated daily with immunosuppressant. The per cent inhibition of the control response is shown for methotrexate (○), CP-17,193 (●), cyclophosphamide (△), 6-mercaptopurine (▲), azathioprine (□) or oxisuran (■).

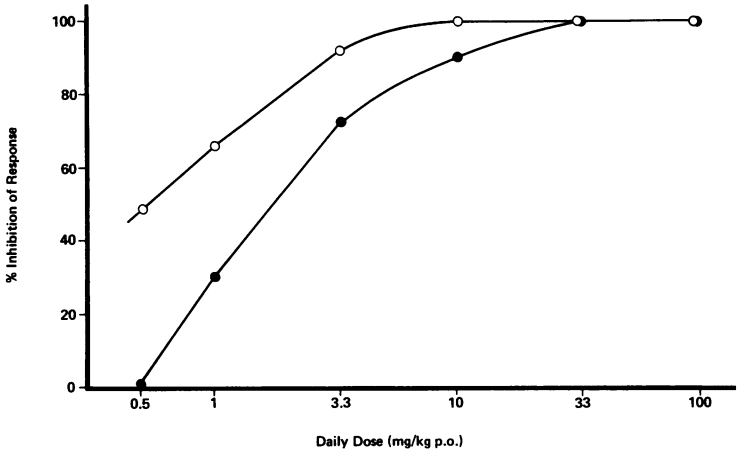


**Fig. 3.** The effect of CP-17,193 on the cellular and humoral response to $EL_4$ cells. Groups of 10 BALB/c mice were immunized with $1 \times 10^8$ $EL_4$ cells on day 0 and on day 10 their cellular and humoral immune response was determined as described under Materials and Methods. Each group was treated from day 0 to day 9 with CP-17,193 or saline. Inhibition of the cellular (●) and the humoral (○) immune response is shown.

respectively. Methotrexate was significantly more potent than CP-17,193 with 50% inhibition at about 0·35 mg/kg p.o.

*Selectivity for the humoral and cellular response.* The relative selectivity of immunosuppression for the humoral cellular immune response was determined using the $EL_4$ cell system described by Otterness & Chang (1976). The results are shown in Fig. 3. At low doses, CP-17,193 showed a more complete suppression of the humoral response than of the cellular response. At high doses, 10 mg/kg p.o. or more, no selectivity was observed as both responses were totally suppressed.

*Inhibition of the SRBC plaque assay.* The potency of each of the immunosuppressives was examined in the Jerne plaque assay. The rank order of the compounds in the $EL_4$ assay was roughly maintained in the plaque assay (Fig. 4), but the dose required for inhibition was shifted upward in the plaque assay. For example, methotrexate had an $ED_{50}$ of 0·35 mg/kg in the $EL_4$ system but required 1·5 mg/kg for the plaque assay. The $ED_{50}$ for CP-17,193 increased from 1·5 to about 5,

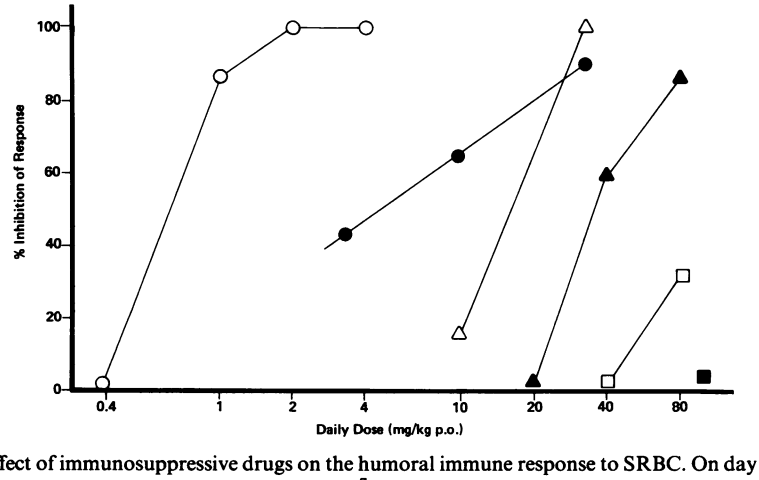


**Fig. 4.** The effect of immunosuppressive drugs on the humoral immune response to SRBC. On day 0, groups of six mice were immunized intraperitoneally with $2 \times 10^7$ SRBC. Immunosuppressants were dosed daily from day 0 to day 5. On day 5, the mice were killed and the Jerne plaque assay used to determine the response. Per cent inhibition of the control response is shown for methotrexate (○), CP-17,193 (●), cyclophosphamide (△), 6-mercaptopurine (▲), azathioprine (□) and oxisuran (■).

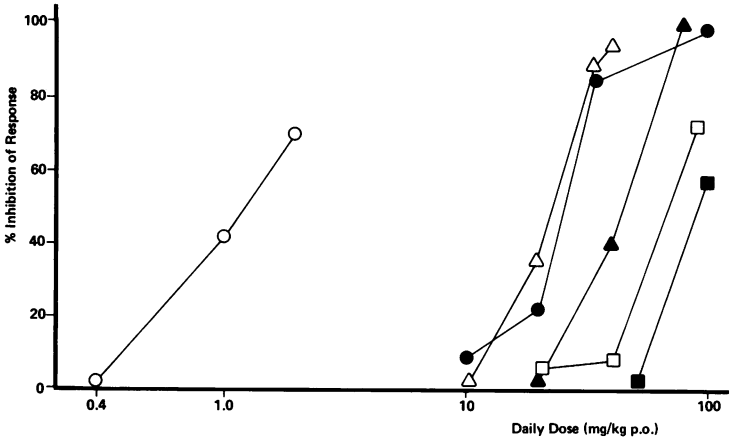


**Fig. 5.** The effect of immunosuppressive drugs on the cellular response to SRBC. On day 0, $1 \times 10^7$ SRBC were injected subcutaneously. Groups of five mice were treated daily with saline or immunosuppressant from day 0 to day 5. The mice were challenged by the subplantar route with $1 \times 10^8$ SRBC and the footpad swelling determined 24 hr later. Per cent inhibition of the control response is shown for methotrexate (○), CP-17,193 (●), cyclophosphamide (△), 6-mercaptopurine (▲), azathioprine (□) and oxisuran (■).

cyclosphosphamide from 10 to 15, 6-mercaptopurine from 8 to 25 and azathioprine from 20 to > 80 mg/kg p.o.

*Inhibition of the SRBC delayed response.* The potency of each of the immunosuppressants in the delayed SRBC response was determined by the footpad assay of Miller *et al.* (1973). The potency of immunosuppression in this assay (Fig. 5) appeared to parallel that of the SRBC plaque assay (Fig.

**Table 1.** Haemagglutination suppressive dose and therapeutic index

| Drug | Results | | | | | Therapeutic index |
|---|---|---|---|---|---|---|
| Azathioprine (mg/kg p.o.) | 0 | 40 | 80 | 160 | 320 | |
| Agglutination inhibition* | 0/5 | 0/5 | 2/5 | 3/5 | 1/1 | |
| Deaths† | 0/5 | 0/5 | 0/5 | 0/5 | 4/5 | 4 |
| Cyclophosphamide (mg/kg p.o.) | 0 | 10 | 20 | 40 | 80 | |
| Agglutination inhibition | 0/5 | 0/5 | 4/5 | 5/5 | 5/5 | |
| Deaths | 0/5 | 0/5 | 0/5 | 1/5 | 3/5 | 2 |
| 6-Mercaptopurine (mg/kg p.o.) | 0 | 20 | 40 | 80 | 160 | |
| Agglutination inhibition | 0/5 | 0/5 | 2/5 | 4/5 | 1/1 | |
| Deaths | 0/5 | 0/5 | 0/5 | 0/5 | 4/5 | 4 |
| Methotrexate (mg/kg p.o.) | 0 | 0.3 | 1.0 | 2.0 | 4.0 | |
| Agglutination inhibition | 0/5 | 2/5 | 4/4 | 4/4 | 1/1 | |
| Deaths | 0/5 | 0/5 | 1/5 | 1/5 | 4/5 | 3 |
| CP-17,193 (mg/kg p.o.) | 0 | 20 | 40 | 100 | 400 | |
| Agglutination inhibition | 0/5 | 0/5 | 5/5 | 5/5 | n.d. | |
| Deaths | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | > 10 |

* Fraction of mice showing agglutination inhibition of greater than two-fold by titre.

† Fraction of mice dying from drug within 15 days.

4) for azathioprine and 6-mercaptopurine. The responses of cyclophosphamide and CP-17,193, however, were both shifted to higher doses. The $ED_{50}$ changed from around 5 to about 25 mg/kg p.o. for CP-17,193. For cyclophosphamide, the $ED_{50}$ changed from about 15 to 23 mg/kg p.o. Oxisuran also showed activity at 100 mg/kg p.o. (56% inhibition) whereas it had been inactive in the plaque assay. The effects of methotrexate were very inconsistent and its $ED_{50}$ varied over a three-fold range from experiment to experiment with both the PFC and the delayed response. The $ED_{50}$'s, however, showed a tendency to be higher for the cellular than the humoral response as illustrated in Figs 4 and 5.

*Haemagglutination and acute therapeutic index measurements.* The method of Berenbaum & Brown (1964) was utilized to obtain a rough measure of immunosuppressive efficacy in the haemagglutination assay. The mice were observed up to day 15 in order to determine toxicity. The results are shown in Table 1. For most of the agents, the doses required to inhibit haemagglutination were comparable to those required to inhibit the SRBC delayed hypersensitivity assay, e.g. azathioprine, cyclophosphamide, 6-mercaptopurine, methotrexate and CP-17,193 required 80, 20, 40, 0·3 and 40 mg/kg p.o. respectively. These doses were compared with the lowest dose giving at least one death to compute an approximate therapeutic index. The therapeutic indices were: azathioprine ($\sim 4$), cyclophosphamide ($\sim 2$), 6-mercaptopurine ($\sim 4$), methotrexate ($\sim 3$) and CP-17,193 ($> 10$).

## DISCUSSION

In a previous communication (Otterness & Chang, 1976), four immunosuppressants—azathioprine, methotrexate, 6-mercaptopurine and cyclophosphamide—were examined for their ability to inhibit the cellular and humoral response to $EL_4$ cells. When CP-17,193 was compared to these agents and oxisuran in the assay of cellular cytotoxicity to $EL_4$ cells, it was found to be a significantly more potent immunosuppressant than azathioprine, 6-mercaptopurine, cyclophosphamide and oxisuran. Methotrexate was the most potent of the agents studied. Oxisuran failed to inhibit either the cellular or humoral response to $EL_4$ cells at 100 and 200 mg/kg p.o.

When the selectivity of CP-17,193 was examined by concurrent measurement of the cellular and humoral response, clear evidence of selectivity was observed. At all the lower effective doses, 0·5–10 mg/kg p.o., a greater inhibition of the humoral response was found. Above 10 mg/kg complete suppression of both the humoral and cellular response was found and all selectivity was lost. Thus, like cyclophosphamide (Otterness & Chang, 1976), CP-17,193 shows no absolute specificity; specificity is partial at the lower active doses where there is weaker, but parallel, inhibition of the cellular response.

The lack of inhibitory activity of oxisuran on the $EL_4$ cellular response was surprising since it was reported to have cellular specificity in a mouse allograft system (Freedman *et al.*, 1972). One possible explanation (Van Dijk *et al.*, 1975) is that oxisuran acts through stimulation of the adrenal cortex. This would be consistent with the data of Fox, Gingold & Freeman (1973), which demonstrates that inhibition of delayed skin tests in rodents is most pronounced when drug is administered on the day preceding skin testing, and with our unpublished data which shows that inhibition of the immune response in the $EL_4$ system is not seen even with high doses of steroid, i.e. 500 mg/kg hydrocortisone. Alternatively, it is possible that oxisuran acts only on the T cell population that mediates delayed hypersensitivity. This T cell population has been shown to be distinct from the population that mediates cellular cytotoxicity; the former is Ly $1^+, 2^-$, the latter Ly $1^-, 2^+$ (Huber *et al.*,1976). Moreover, the fact that oxisuran inhibits the development *de novo* of delayed skin tests but does not inhibit the expression of established delayed tests in man (Pirofsky, Nolte & Bardana, 1975) also suggests that the effects of oxisuran may not be due solely to the anti-inflammatory effects of adrenal stimulation.

The $EL_4$ cell system measures the induction of cytotoxicity across the major H-2 histocompatibility difference. Differences in major histocompatibility loci lead to strong mixed lymphocyte reactions, good generation of cellular cytotoxicity (Bach, Bach & Sandel, 1976), a uniquely high portion of responding lymphocytes, and a failure to mount an enhanced secondary response

(Simonsen, 1970). Thus it can be argued that the $EL_4$ system might be unique and not reflect results obtained with a more typical antigen. Therefore, immunosuppression of the humoral and cellular responses were measured with a second antigenic system, SRBC. The SRBC system was chosen because of its requirements for both T cell help (Mitchell & Miller, 1968) and for accessory cells (Mosier, 1967) in the induction of the response. Further, unlike the $EL_4$ cell system for which the major H-2 differences are strongly linked with T cell mitogenicity, the SRBC has been considered to be a weak polyclonal B cell mitogen (Coutinho & Möller, 1975).

In experiments examining the plaque response and the delayed skin test toward SRBC, azathioprine and 6-mercaptopurine were again non-selective agents. Methotrexate exhibited some selectivity for the humoral response but oxisuran inhibited only the cellular response. It failed to inhibit the plaque assay at all doses tested. Both cyclophosphamide and CP-17,193 maintained their selectivity for the humoral response. The major difference observed between the two systems does not appear to be one of selectivity, but of dose dependency; it took far more immunosuppressive drug to inhibit the anti-SRBC response than the anti-$EL_4$ response. The difference in suppressive dose required in the two antigenic systems shows that care must be taken to use the same antigen for both humoral and cellular assays. In this work, three to 10 times less immunosuppressive drug was required to inhibit the response to $EL_4$ than that to SRBC. Thus, if one antigen is used to measure the cellular response and another antigen is used to measure the humoral response, erroneous conclusions about specificity may result.

A second important point emerges from these measurements of the dose of immunosuppressant required to inhibit the immune response. Different amounts of immunosuppressive were required to inhibit the response to each antigen. This in turn results in a different therapeutic index for each antigen. For example, the acute therapeutic index for cyclophosphamide appears poor for inhibition of haemagglutinin formation, yet it is quite tolerable when calculated for the $EL_4$ cell system. Thus, the safety and, therefore, utility of immune suppressants may in fact depend heavily on the nature of the antigen. Clinically this may be reflected in the requirement for grafting across weak rather than strong histocompatibility loci. The nature of the antigen may, in the final analysis, serve to determine whether new immunosuppressants can be useful in autoimmune states. As with cyclosphosphamide in this study, immunosuppressants might well show therapeutic effects with a good margin of safety against certain antigens, whereas against other antigens, an immunosuppressant might be effective only at overtly toxic doses.

Finally, although the mode of action of CP-17,193 is not yet defined, its structural dissimilarity from known antimetabolites and alkylating agents would suggest it may exert its influence on the immune response by a unique mechanism. At present there is some evidence to support this contention. Whereas immunosuppressant drugs like methotrexate and azathioprine inhibit lymphocyte proliferation in tissue culture, CP-17,193 not only does not inhibit, but acts as a stimulant of lymphocyte proliferation in the tissue culture system of Otterness and co-workers (Otterness, Bliven & Holden, 1979; Otterness, Holden & Bliven, in preparation). Thus, in addition to being a potent and selective immunosuppressant, CP-17,193 may serve as a useful tool for exploring the requirements for elicitation of the immune response.

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## REFERENCES

BACH, F.H., BACH, M.L. & SANDEL, P.M. (1976) Differential functions of major histocompatibility complex antigens in T-lymphocyte activation. *Nature*, **259**, 273.

BERENBAUM, M.C. & BROWN, I.N. (1964) Dose response relationships for agents inhibiting the immune response. *Immunology*, **7**, 65.

COUTINHO, A. & MÖLLER, G. (1975) Thymus-independent B-cell induction and paralysis. *Adv. Immunol.* **21**, 113.

CUNNINGHAM, A. & SZENBERG, A. (1968) Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology*, **14**, 599.

FOX, A.E., GINGOLD, J.L. & FREEMAN, H.H. (1973) Inhibition by oxisuran of cell-mediated hypersensitivity by decrease in numbers of specifically sensitized cells. *Infect. Immun.* **8**, 549.

FREEDMAN, H.H., FOX, A.E., SHAVEL, J., JR & MORRISON, G.C. (1972) Oxisuran: a differential inhibitor

of cell-mediated hypersensitivity. *Proc. Soc. exp. Biol. Med.* **139,** 909.

HUBER, B., CANTOR, H., SHEN, F.W. & BOYSE, E.A. (1976) Independent differentiative pathways of Ly 1 and Ly 2,3 subclasses of T-cells. Experimental production of mice deprived of selected T-cell subclasses. *J. exp. Med.* **144,** 1128.

KABAT, E.A. & MEYER, M.M. (1961) *Experimental Immunochemistry* 2nd edn, p. 136. Charles C. Thomas, Springfield, Illinois.

LOMBARDINO, J.G. & OTTERNESS, I.G. (1981) Novel immunosuppressive agents. Potent immunological activity of some benzothiopyrano (4,3-*c*) pyrazol-3-ones. *J. Med. Chem.* **24,** 830.

MILLER, T.E., MACKANESS, G.G. & LAGRANGE, P.H. (1973) Immunoprotection with BCG. II. Modulation of the response to sheep red blood cells. *J. Natl. Cancer Inst.* **51,** 1969.

MITCHELL, G.F. & MILLER, J.F.A.P. (1968) Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. *J. exp. Med.* **128,** 821.

MOSIER, D.E. (1967) A requirement for two cell types for antibody formation *in vitro*. *Science*, **158,** 1573.

OTTERNESS, I.G., BLIVEN, M.L. & HOLDEN, H.E., JR (1979) Effects of levamisole on the mitosis of murine thymocytes in culture. *Immunopharmacology*, **1,** 245.

OTTERNESS, I.G. & CHANG, Y.-H. (1976) Comparative study of cyclophosphamide, 6-mercaptopurine, azathioprine and methotrexate. Relative effects on the humoral and cellular immune response in the mouse. *Clin. exp. Immunol.* **26,** 346.

PIROFSKY, B., NOLTE, M.T. & BARDANA, E.J., JR (1975) The effect of oxisuran on human immunological responsiveness. *Transplantation*, **20,** 357.

SIMONSEN, M. (1970) On the nature and measurement of antigenic strength. *Transplant. Rev.* **3,** 22.

VAN DIJK, H., BAKKER, I.A., TESTERINK, J., BLOKSINA, N. & WILLERS, J.M. (1975) Oxisuran and immune reactions: mediation of oxisuran action via the adrenal glands. *J. Immunol.* **115,** 1587.