THE EFFECT OF QUADRUPLE CHEMOTHERAPY ON THE PERIPHERAL BLOOD LYMPHOCYTE POPULATIONS IN HODGKIN'S DISEASE

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- 1 Quadruple chemotherapy with cytotoxic drugs in advanced (Stage IIIB) Hodgkin's disease causes a fall in the blood lymphocyte count with recovery 1 month after stopping treatment.
- 2 No selective effect on the T or B lymphocyte populations was shown.

Introduction

There is now considerable evidence that cellular immunity modifies the course of Hodgkin's disease (Chawla, Stutzman, Dubois, Kim & Sokal, 1970; Sokal & Aungst, 1969). It would therefore be expected that any factors which suppress cellular immunity might favour extension of the disease. Cellular immunity is mediated by thymus derived lymphocytes (T lymphocytes) in contradistinction to humoral immunity in which B lymphocytes play the chief role (Roitt, Torrigiani, Greaves, Brostoff & Playfair, 1969).

Most cytotoxic drugs decrease the number of circulating lymphocytes and neutrophils. In view of the increasing use of cytotoxic drugs in malignant disease, particularly in the lymphomas, it is of relevance to know whether the drugs commonly used reduce the circulating lymphocyte population, how long this decrease persists, and whether there is any selective depression of T or B lymphocytes.

A preliminary report of this work was presented to the British Pharmacological Society Meeting in December 1975 (Houlihan, Swain & Trounce, 1976).

Methods

Treated patients

Six patients with Stage IIIB Hodgkin's disease were studied; five of them had been staged by laparotomy and their spleens had been removed. Two patients had received radiotherapy 1 year prior to the study. Otherwise no patient had received any specific treatment.

Control patients

- (a) Six healthy adults of similar age to the patients receiving treatment.

- (b) Four patients with Stage IIIB Hodgkin's disease undergoing pretreatment investigation.

Treatment

All treated patients received the standard MVPP regime, i.e., mustine 6 mg/m² i.v., Days 0 and 7; vinblastine 10 mg i.v., Days 0 and 7; procarbazine 100 mg orally daily, Days 1 to 14; prednisolone 40 mg orally daily, Days 1 to 14. Total white count was measured by Coulter counter. Differential counts were prepared on a May-Grunwald-Giemsa preparation. Lymphocytes were separated from heparinized blood on a Ficoll/Trisoll density gradient (Boyum, 1968). T lymphocytes were identified by rosette formation with sheep red blood cells (Jondal, Holm & Wigzell, 1972). B lymphocytes were identified by immunofluorescent staining of surface immunoglobulins (Papamichail, Brown & Holborow, 1971). The patients receiving treatment were studied through their first cycle of MVPP cytotoxic regime.

The total white count, the differential count and the numbers of T and B lymphocytes in the peripheral blood were counted before treatment and at 7–14 and 42 days after the commencement of treatment.

The results were compared with counts in healthy controls receiving no drugs and with a group of patients with Hodgkin's disease of the same stage who were receiving no treatment.

Results

These are shown in Figures 1 and 2.

In the patients receiving cytotoxic drugs:

- (a) The total white cell count fell between days 0 and 14.
- (b) The numbers of lymphocytes fell significantly

Figure 1 The total white blood count (mean $\pm$ s.e. mean) during and following one cycle of MVPP treatment. ● controls; ○ treated patients; ■ untreated patients.

between days 0 and 7 but returned to pretreatment levels by day 42.

- (c) The fall in lymphocytes included both T and B cells and there was no evidence of selective depression of either lymphocyte subpopulation.

In the control groups, the counts showed no significant change except for a just significant fall of T lymphocytes in healthy adults between days 7 and 42.

Discussion

It is clear that the MVPP regime when used in treating Hodgkin's disease produces a considerable lymphopenia, the peripheral blood count falling to about one third the pretreatment level. It seems most likely that this is due to a direct mitostatic effect on the cells and there is evidence that alkylating agents will produce this effect in animals (Stockman, Heim, South & Trentin, 1973; Turk & Poulter, 1972). A possible contributing factor is the shift of lymphocytes from the peripheral circulation to the bone marrow, this phenomena has been described as a consequence of

steroid administration in animals (Moorhead & Claman, 1972; Fauci, 1975) and in man (Fauci & Dale, 1974). However, it appears that in contradistinction to our findings, this shift involves predominantly, though not entirely, T lymphocytes.

The lymphopenia occurring with treatment, and the rapid recovery after treatment has stopped would suggest that the mitostatic action of the drugs is suppressing a subpopulation of lymphocytes which are rapidly dividing and short-lived. Whether a similar response to cytotoxic drugs would be found in normal subjects is impossible to say. There is, however, considerable evidence of disturbed lymphocyte morphology and function in Hodgkin's disease (Aisenberg, 1965; Crowther, Fairley & Sewell, 1969) and it may be that in this disease there is a high proportion of young lymphocytes, which are particularly sensitive to cytotoxic drugs. Alternatively, circulating lymphocytes may have already been damaged by circulating lymphotoxic substances which have been reported from time to time (Scheurlen, Schneider & Pappas, 1971) and are thought to be produced as a result of the disease process.

Whatever the mechanism of the lymphopenia, recovery is rapid and the peripheral count of both T and B lymphocytes has returned to normal within a few weeks. This differs from the results of radiotherapy where it has been reported (Stjernsward, Jondal, Vanky, Wigzell & Sealy, 1972) that following a course of treatment for breast cancer the lymphocyte count may be depressed for up to 1 year and that there is a predominant reduction in T lymphocytes.

It is possible that repeated courses of treatment with cytotoxic drugs might produce a more prolonged lymphopenia. This has not been studied here, but previous work (Swain & Trounce, 1974) in which total and T lymphocytes were studied in patients with very advanced Hodgkin's disease throughout six cycles of MVPP treatment showed no fall in peripheral lymphocytes and a slight rise in T cell population. It must be admitted, however, that in this series the initial lymphocyte counts were low, probably as a result of the disease itself.

The MVPP regime does not appear to selectively depress T or B lymphocytes. This is perhaps a little surprising as steroid hormones which are a part of the MVPP combination have been shown to reduce peripheral T lymphocytes in man (Fauci & Dale, 1974). However, it has also been reported that cyclophosphamide, which is very similar to mustine, depletes predominantly the B lymphocytes areas in the experimental animal (Turk & Poulter, 1972). It is important to remember that in the present investigation several drugs were used in a rather complex fashion in contradistinction to the single drug and often single injection experimental model. Also that the disease process itself may well modify both the lymphocytes and their responses.

The most important practical aspect of depression

Figure 2 (a) Total lymphocytes and (b) numbers of T & B cells during and following one cycle of MVPP treatment. ● controls; ○ treated patients.

of white cells is whether this increases the risk of intercurrent infections.

With lymphopenia the risks are hard to assess, particularly as in Hodgkin's disease the pathological process itself can be shown to depress some aspects of cellular immunity (Lamb, Pilney, Kelly & Good, 1962; Green & Corso, 1959). It is well known that patients with Hodgkin's disease are particularly susceptible to infection including that due to viruses and fungi (Casazza, Duvall & Carbone, 1966). That combination chemotherapy may increase the incidence of viral infection is suggested by a report that the incidence of herpes zoster was roughly doubled in patients receiving combined chemotherapy (Goffinet, Glatstein & Merigan, 1972).

Even more difficult to assess is the effect of lymphopenia on the disease process itself. Lymphopenia in Hodgkin's disease is associated with widespread disease (Brown, Haynes, Foley, Godwin, Berard & Carbone, 1967) and lymphocyte depletion

in the affected lymph nodes carries a worse prognosis than lymphocyte predominance. However, there is good evidence that whatever the transient effects of MVPP treatment on lymphocytes may be, the ultimate results in terms of morbidity and mortality overwhelmingly support the use of combinations of cytotoxic drugs in the appropriate patients (de Vita, Serpick & Carbone, 1970).

It is possible that in some patients recovery of cell mediated immunity after treatment may play an important part in combating or eradicating small remaining areas of disease. If this is so, the rapid recovery of lymphocyte blood levels after stopping cytotoxic drugs is encouraging. It may be that the new lymphocytes appearing after cytotoxic treatment are not functionally competent and this raises the difficult and as yet unsolved problem of testing for immune competence against cancer. The correlation, if any, between various tests of lymphocyte function and the ability of lymphocytes to attack and destroy

maligant cells is not known. Until such knowledge is available it is only possible to say that the above investigation suggests that the MVPP regime does not

produce a prolonged depression of T or B lymphocytes in the peripheral blood, and in terms of numbers of cells immune competence should rapidly recover.

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