Eur J Cancer Clin Oncol, Vol. 20, No. 1, pp. 91–98, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Combination Chemotherapy and Immune Capacity in Advanced Ovarian Carcinoma†

R. J. M. TEN BERGE,†‡ P. TH. A. SCHELLEKENS,† J. V. T. H. HAMERLYNCK§ and P. F. BRUNING§

†*Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam and §The Netherlands Cancer Institute, Antoni van Leeuwenhoekziekenhuis, Amsterdam, The Netherlands*

Abstract—*The effects of chemotherapy with either Chap-5 (a drug regimen consisting of adriamycin,* cis-*dichlorodiammine platinum (II), hexamethylmelamine and cyclophosphamide) or Hexa CAF (a drug regimen consisting of methotrexate, 5-fluorouracil, hexamethylmelamine and cyclophosphamide) on the immunocompetence of 22 patients with advanced ovarian carcinoma were studied. Both primary and secondary humoral and cellular immune responses* in vivo *were studied. In addition, the numbers of granulocytes, lymphocytes and monocytes in peripheral blood were determined, as well as the levels of immunoglobulins and complement proteins. Furthermore, the proliferative capacity of lymphocytes, cytotoxic T-cell function, and K- and NK-cell activities were measured. The results indicate a depression of the primary humoral immune response* in vivo *in patients receiving Chap-5. Furthermore, a decrease of several parameters* in vitro *was observed. However, these alterations were only moderate and rapidly reversible.*

INTRODUCTION

PREVIOUS studies have considered the effects of chemotherapy on either cellular or humoral immune reactivity in man [1–6]. The drugs methotrexate and cyclophosphamide are known to exert immunosuppressive effects [1, 7]. On the other hand, adriamycin appears to have minimal effects on cellular immunity *in vivo* and *in vitro* [3]. However, only limited data are available on the effects of drug regimens, which include *cis*-dichlorodiammine platinum (II) (*cis*platin) [8–12] and hexamethylmelamine [9]. Because especially *cis*platin seems to be a useful agent in the treatment of several human malignancies [13, 14], it is important to characterize the effect of drug regimens, including this drug, on the immune capacity of man.

The purpose of the present study was to determine whether chemotherapy with Chap-5 (adriamycin, *cis*platin, hexamethylmelamine and cyclophosphamide) or with Hexa CAF (methotrexate, 5-fluorouracil, hexamethylmelamine and cyclophosphamide) altered the primary and secondary humoral and cellular immune responses as measured *in vivo* and *in vitro*. At the same time, this study will provide information on the effect of these drugs on the defence mechanisms in cancer patients.

Accepted 30 June 1983.

*The investigations were supported in part by the Foundation for Medical Research FUNGO, which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

‡Correspondence and reprint requests to: R. J. M. ten Berge, c/o Publication Secretariat, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, P.O. Box 9406, 1006 AK Amsterdam, The Netherlands.

MATERIALS AND METHODS

Patients

Twenty-two patients, treated in the Netherlands Cancer Institute (Antoni van Leeuwenhoekziekenhuis, Amsterdam) for epithelial ovarian tumours [International Federation of Gynaecology and Obstetrics (FIGO) classification stage III or IV], were included in this study. From all patients informed consent was obtained. At least 3 weeks before randomization to chemotherapy, a laparotomy had been performed to remove as much tumour as technically possible (including the uterus, ovaries and omentum). The patients had not previously received radiotherapy or chemotherapy. The mean age was 54 years (range 42–67).

Drug treatment

Sixteen patients had been randomized to treatment with Chap-5. This drug regimen was as follows: during the first part of the cycle, adriamycin at a dose of 35 mg/m^2 was given i.v. on day 1, and *cis*platin at a dose of 20 mg/m^2 was given i.v. on days 1–5. In the second part of the cycle, from day 15 onwards, hexamethylmelamine at a dose of 150 mg/m^2 and cyclophosphamide at a dose of 100 mg/m^2 were given orally on days 15–28. From day 29 to day 35 no drugs were given, and then the cycle was repeated.

Six patients had been randomized to treatment with Hexa CAF. Their drug regimen was as follows: methotrexate at a dose of 40 mg/m^2 and 5-fluorouracil at a dose of 600 mg/m^2 were given i.v. on days 1 and 8, and hexamethylmelamine and cyclophosphamide were each given orally at a dose of 150 mg/m^2 on days 1–14. From day 15 to day 28 no drugs were given, and then the cycle was repeated.

Design of the study

Following the first blood collection before initiation of chemotherapy, all patients were studied during the first cycle. Because the time interval between administration of the drug and antigenic challenge can markedly influence the outcome of any resulting immune reponse, the patients were immunized at different time-points during the cycle of chemotherapy. Therefore, they were randomized into 3 groups: group A consisted of 8 patients receiving Chap-5; group B comprised another 8 patients receiving Chap-5; and group C consisted of 6 patients receiving Hexa CAF.

The patients in group A were immunized on the second day of the cycle, i.e. during therapy with *cis*platin. The patients in group B were immunized on day 22 of the cycle, i.e. while on therapy with hexamethylmelamine and cyclophosphamide. The patients in group C were immunized on day 15 of the cycle, i.e. just after completion of the course of hexamethylmelamine and cyclophosphamide, and on the first drug-free day of the cycle. The whole scheme is presented in Fig. 1.

After collection of blood, each patient was immunized s.c. with 1.0 mg haemocyanin from α-helix pomatia to elicit a primary humoral immune response, and with a patch containing 1 mg DNCB to elicit a primary cellular immune response. Secondary humoral immune responses were evoked by immunization with 1 ml of alum-precipitated DTP vaccine, containing diphtheria toxoid (2.5 Lf), tetanus toxoid (5 Lf) and inactivated polio virus, types I, II and III. After 13 days, blood was drawn again and skin tests were performed with dinitrochlorobenzene (DNCB) and, to test secondary cellular immune responses, with a variety of recall antigens [purified protein derivative of tuberculin (PPD), varidase, mumps, trichophyton and candida], injected i.d. Skin tests were read 48 hr after challenge.

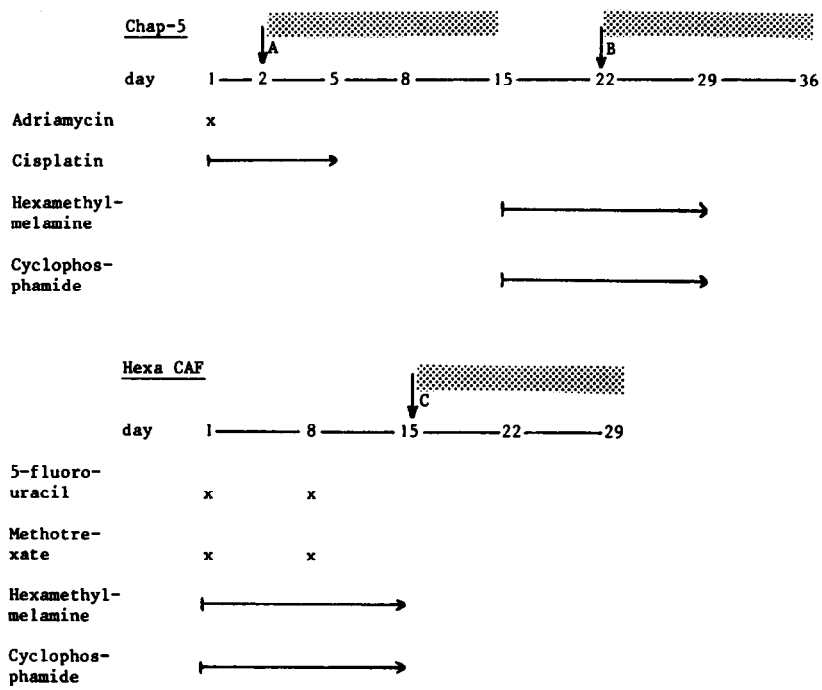

Fig. 1. Schematic representation of the studied drug regimens. $\downarrow^A$ ▒ *Time-period of immunological studies in group A.* $\downarrow^B$ ▒ *Time-period of immunological studies in group B.* $\downarrow^C$ ▒ *Time-period of immunological studies in group C.*

Blood samples

Serum obtained from each individual was frozen and kept at -20°C. Mononuclear cells were isolated from defibrinated blood by Ficoll-Isopaque density-gradient centrifugation and preserved in liquid nitrogen [15]. After thawing, lymphocyte cultures of one patient, obtained before and twice during the first cycle of chemotherapy, were performed in one experiment on the same day, thus avoiding day-to-day variations in the lymphocyte cultures.

Cell numbers in peripheral blood

The absolute numbers of granulocytes, lymphocytes and monocytes in peripheral blood were determined by electronic cell counting (Coulter counter) and differentiation in May-Grünwald-Giemsa-stained blood smears. T lymphocytes were scored by rosette formation with sheep erythrocytes. B lymphocytes were identified by a direct immunofluorescence test using $F(ab')_2$ fragments of sheep-anti-human Ig serum conjugated to fluorescein isothiocyanate (FITC).

Serum proteins

The total serum-protein concentration and serum-protein spectrum were determined by standard methods. The levels of IgG, IgM and IgA were determined by a nephelometric technique, and those of IgD and IgE by a radioimmunoassay. The levels of complement components were determined by radial immunodiffusion.

Specific humoral immune responses

Specific antibodies of the IgM, IgG and IgA classes to the antigen haemocyanin, as well as total antibody levels against diphtheria toxoid, were measured by an enzyme-linked immunosorbent assay (ELISA) technique. Total antibody and IgG-class antibody levels against tetanus toxoid were determined by a radioimmunoassay. Anti-polio-virus antibodies were measured by virus neutralization.

Cellular immune reactivity in vitro

In-vitro *lymphocyte transformation tests.* After thawing, the viability of the cell suspensions used for the experiments was determined by trypan-blue exclusion and found to be always higher than 95%. Cultures were performed in microtitre plates [16], containing 3×10^4 lymphocytes per well in 150 µl; the following stimulants were used: (1) the non-specific mitogens PHA (Wellcome), final concentration 50 µg/ml; horse-anti-human ALS, final dilution 1:34; ConA (Sigma), final concentration 120 µg/ml; PWM (Gibco), final concentration 100 µg/ml; (2) the antigen α-helix pomatia haemocyanin, final concentration 50 µg/ml; (3) an antigen cocktail, consisting of a mixture of the following antigens: PPD, final concentration 100 µg/ml; varidase, final concentration 100 E/ml; mumps, final concentration 0.08 CFU/ml; trichophyton, final concentration 2%; candida, final concentration 1:200 [17]; and (4) 3×10^4 lymphocytes, irradiated at a dose of 2000 rad to test the responder capacity in MLC (mixed lymphocyte culture).

Responses to PHA, ALS and ConA were assessed after 3 days of culture, responses to PWM and microbial antigens after 6 days of culture; mixed lymphocyte cultures were performed for 6 days. Twenty-four hours before termination of the cultures, [^{3}H]thymidine was added (0.8 µCi per well; specific activity 400 Ci/mol). On each occasion when the assays were performed, cryopreserved lymphocytes from healthy donors were tested in parallel. The results obtained from these control lymphocyte cultures were consistently within the same, normal range.

Cytotoxicity assays. The effector function of lymphocytes was assessed by different cytotoxicity assays. In the cell-mediated lympholysis (CML) test [18], the cytotoxic capacity of MLC-activated T lymphocytes (CTL, cytotoxic T lymphocytes) was tested against peripheral blood lymphocytes from two unrelated healthy individuals. In the antibody-dependent lympholysis (ADL) test, the capacity of the K cells in the lymphocyte suspensions to lyse ^{51}Cr-labelled mouse-P815-mastocytoma cells sensitized with an IgG rabbit-anti-mastocytoma antibody was measured [19]. The function of NK cells was assessed in a 4-hr ^{51}Cr-release assay, using K-562 cells as target cells [20]. All cytotoxicity assays were performed in triplicate at 6 different effector-cell concentrations and a constant number of target cells.

Cellular immune reactivity in vivo

Sensitization to DNCB was tested after 14 days with two concentations of DNCB (3 and 10 µg per patch). Forty-eight hours later, the test was evaluated as follows:

	Score
Erythema	1
Erythema and induration	2
Erythema, induration and blistering	3
Erythema, induration, blistering and ulcer	4

The final DNCB score was calculated as the sum of the scores of each patch. Delayed-type hypersensitivity to recall antigens was determined by skin tests using 5 different antigens: PPD (10 TU/ml), varidase (50 U SK/SD/ml), mumps (20 CFU/ml), trichophyton (0.1%) and candida

(1:300). Each antigen solution (0.1 ml) was injected i.d. on the volar surface of the forearm. An induration of 5 mm or more at two perpendicular diameters after 48 hr was considered positive.

Statistical methods

Cell numbers in peripheral blood, the levels of albumin, IgM, IgG and IgA, and of complement components were tested by analysis of variance. Levels of IgD and IgE, antibody levels as well as the results of lymphocyte cultures were tested by analysis of variance after logarithmic transformation of the individual values. Results of lymphocyte cultures were also tested by Wilcoxon's signed rank test. Cytotoxicity assays were tested by regression analysis. All tests were two-tailed.

RESULTS

Cell numbers in peripheral blood

Table 1 shows the absolute numbers of granulocytes, lymphocytes, T and B lymphocytes and monocytes. In each group of patients, the number of granulocytes was depressed, at least from day 15 of the first cycle of chemotherapy. The total number of lymphocytes (both T and B lymphocytes) showed a decrease only after a 14-day course of cyclophosphamide and hexamethylmelamine (group C); in the subsequent therapy-free period, the lymphocyte counts recovered. The number of monocytes showed no significant decrease.

Table 1. Cell counts in peripheral blood

	Group A (Chap-5, day 2)			Group B (Chap-5, day 22)			Group C (Hexa CAF)			
	Before	Day 2 after initiation of therapy	Day 15 after initiation of therapy	Before	Day 22 after initiation of therapy	Day 35 after initiation of therapy	Before	Day 15 after initiation of therapy	Day 28 after initiation of therapy
No. of granulocytes $\times 10^9/l$	6.63 ± 1.27	5.65 ± 2.14	1.73 ± 0.46*†	5.07 ± 2.00	2.00 ± 1.25*	1.61 ± 0.87*	5.37 ± 1.64	1.66 ± 0.36*	2.41 ± 0.63*†
Total No. of lymphocytes $\times 10^9/l$	2.01 ± 1.06	1.92 ± 0.84	1.53 ± 0.77	2.08 ± 1.06	1.49 ± 0.47	1.39 ± 0.51	2.14 ± 0.64	0.55 ± 0.19*	1.52 ± 0.57†
No. of T lymphocytes $\times 10^9/l$	1.50 ± 0.90	1.60 ± 0.77	1.19 ± 0.63	1.46 ± 0.76	1.05 ± 0.27	1.10 ± 0.45	1.51 ± 0.35	0.44 ± 0.15*	1.19 ± 0.51†
No. of B lymphocytes $\times 10^9/l$	0.18 ± 0.10	0.13 ± 0.05	0.13 ± 0.07	0.27 ± 0.16	0.16 ± 0.08	0.13 ± 0.06	0.23 ± 0.08	0.05 ± 0.03*	0.13 ± 0.03†
No. of monocytes $\times 10^9/l$	0.24 ± 0.13	0.20 ± 0.14	0.19 ± 0.03	0.32 ± 0.13	0.26 ± 0.18	0.27 ± 0.13	0.20 ± 0.11	0.12 ± 0.09	0.17 ± 0.06

All values represent the mean ± S.D.
*Significantly decreased as compared to the value before initiation of therapy ($P < 0.05$).
†Significantly different in comparison with the value of day 2 (group A, $P < 0.05$) and day 15 (group C, $P < 0.05$).

Serum proteins

In none of the 3 groups did the levels of albumin, immunoglobulins and complement components show a significant decrease at any time-point (not shown).

Specific humoral responses

In Fig. 2, antibody responses of the 3 groups of patients are shown in comparison with those of a historical control group, measured 13 days after immunization with haemocyanin. (The historical control group consisted of 15 healthy individuals [21].) For obvious reasons, it was not possible to study a non-treated control group of patients with ovarian carcinoma.

The responses in group C were not different from those in the historical control group. However, the responses in group A and even more in group B were decreased in comparison with the responses in group C and in healthy control individuals ($P < 0.05$). In contrast, regarding secondary antibody responses, no differences between the 3 groups of patients were observed, and the responses in each group were normal (not shown).

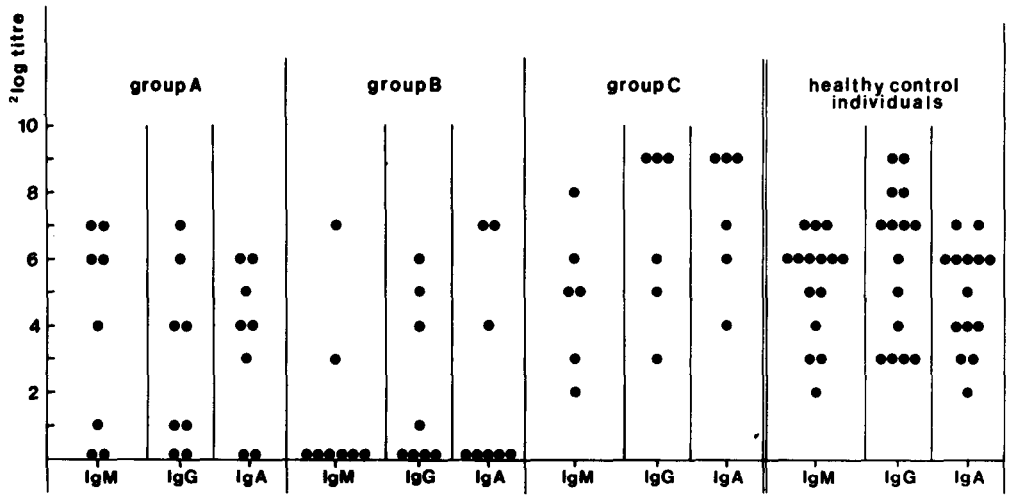

Fig. 2. Primary antibody response 13 days after immunization with hemocyanin. Response are expressed as ^{2}log titre, starting at a dilution of 1:100.

Cellular immune reactivity in vitro

During chemotherapy, the percentage of T lymphocytes in peripheral blood remained constant. Therefore, in cultures performed in this period, the absolute number of T lymphocytes was about the same. The results of the lymphocyte cultures (Table 2) show a moderate, although not significant effect of the cytotoxic drug regimen on the proliferative capacity of lymphocytes from patients in group C, tested at day 15 of the cycle. In group A, a significant decrease of proliferative responses was observed at day 15; in group B, a transient decrease was detected. Table 2 also shows a low reactivity of lymphocytes to specific antigens in all 3 groups of patients, occurring before the initiation of drug therapy. *In vivo* immunization with haemocyanin and DTP vaccine moderately improved these responses.

No alterations were detected in the function of cytotoxic T lymphocytes (CTL), K and NK cells in patients of groups A and B during their first cycle of chemotherapy. However, in patients of group C, CTL function was transiently decreased (mean percentage ^{51}Cr release ± S.D. before drug therapy: 25.7 ± 13.7; at day 15 of the chemotherapy cycle: 7.5 ± 8.0, measured at an effector-target ratio of 5:1; $P < 0.05$). In this group of patients, K and NK cell function showed a slight temporary decrease too, which failed to reach significance (data not shown).

Cellular immune responses in vivo

No differences in reactivity to DNCB were observed between the 3 groups of patients (Fig. 3). Moreover, this reactivity seemed only minimally depressed in comparison with a historical control group of healthy individuals [21].

Finally, the skin test reactivity to anamnestic antigens was depressed in all 3 groups (Fig. 4), which is compatible with the low reactivity *in vitro* after stimulation with the same antigens in the so-called 'antigen cocktail'.

DISCUSSION

This study demonstrates a moderately diminished ability to mount a primary antibody response *in vivo* in patients who were immunized during therapy with adriamycin and *cis*platin (group A). A more severe depression of the primary humoral response was observed in patients who were immunized during therapy with hexamethylmelamine and cyclophosphamide (group B). Apparently normal responses were observed when patients were immunized immediately after a 14-day course of the latter drug regimen (group C). Because all patients were comparable with respect to both severity of disease and the time interval between surgery and initiation of chemotherapy, it is likely that the observed decrease of primary humoral response is caused by the treatment with cytotoxic drugs. Whether adriamycin, *cis*platin, or both drugs are responsible for the decrease observed in group A cannot be decided. Earlier studies have indicated an inhibition of the primary humoral response to sheep erythrocytes after administration of *cis*-platin in mice [22]. In view of the results of a previous study [7], the depressed responses in group B may be caused by treatment with cyclophosphamide. However, a possible effect of hexamethylmelamine cannot be excluded. In view of the only moderate depression of the number of B cells, it may be concluded that these drugs directly affect the function of B cells. Anyway, this effect seems to be rapidly reversible, as appears from the fact that immunization, immediately after cessation of a 14-day course

Table 2. Proliferative capacity of lymphocytes in vitro

	Group A (Chap-5, day 2)			Group B (Chap-5, day 22)			Group C (Hexa CAF)		
	Before	Day 2 after initiation of therapy	Day 15 after initiation of therapy	Before	Day 22 after initiation of therapy	Day 35 after initiation of therapy	Before	Day 15 after initiation of therapy	Day 28 after initiation of therapy
% T-ly	71% ± 9.0	83% ± 9.0	76% ± 9.2	70 ± 15	72 ± 10	69 ± 16	72 ± 10	79 ± 9	77 ± 6
ALS	17.4 ± 6.0	15.3 ± 7.9	10.5 ± 5.4*	15.2 ± 4.7	9.4 ± 3.3*	12.9 ± 7.0	14.0 ± 6.8	8.0 ± 2.9	13.9 ± 6.3
PHA	9.6 ± 5.5	7.6 ± 3.3	4.7 ± 2.0*	8.5 ± 4.4	4.8 ± 1.2*	7.1 ± 2.2	9.4 ± 5.9	10.0 ± 2.5	10.2 ± 5.4
ConA	13.9 ± 6.9	10.8 ± 5.0	10.0 ± 6.4	11.8 ± 6.8	6.6 ± 2.9*	9.9 ± 6.5	13.8 ± 7.8	10.6 ± 3.7	15.6 ± 6.5
PWM	12.9 ± 8.1	10.1 ± 10.2	5.6 ± 2.3*	11.5 ± 7.1	6.4 ± 3.2*	8.0 ± 5.2*	8.0 ± 3.2	6.3 ± 1.9	8.0 ± 1.5
MLC									
Responder capacity to X_R	10.5 ± 5.2	9.7 ± 4.9	8.1 ± 5.2	9.7 ± 4.0	6.1 ± 3.4*	6.9 ± 3.1	11.7 ± 7.5	9.7 ± 9.8	12.3 ± 10.0
to Y_R	6.7 ± 3.9	6.1 ± 4.0	3.7 ± 2.5*	7.2 ± 2.3	5.7 ± 3.7	4.4 ± 1.6	10.2 ± 4.3	7.6 ± 3.2	9.1 ± 5.5
Antigen cocktail	2.10 ± 2.40		1.19 ± 1.27	0.93 ± 0.83		1.71 ± 3.32	2.03 ± 2.18		1.65 ± 1.63
Hemocyanin	0.20 ± 0.13		1.37 ± 1.43	0.12 ± 0.04		0.94 ± 1.28	0.20 ± 0.11		1.55 ± 1.57
Tetanus toxoid	1.35 ± 1.58		1.29 ± 1.43	0.24 ± 0.19		1.13 ± 1.96	0.37 ± 0.32		1.65 ± 1.88
Diphtheria toxoid	0.37 ± 0.28		0.80 ± 0.82	0.12 ± 0.04		0.85 ± 1.05	0.20 ± 0.11		0.73 ± 0.96

All values represent the mean ± S.D., expressed as cpm × 10^{-3} of [^{3}H]thymidine incorporation.
*Significantly decreased in comparison with the value before initiation of therapy ($P < 0.05$).

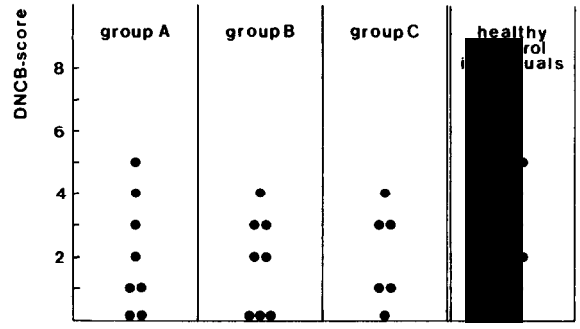

Fig. 3. Primary cellular response in vivo after sensitization with DNCB.

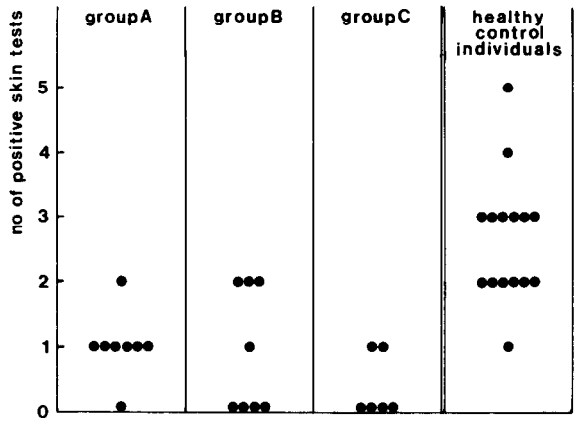

Fig. 4. Secondary DTH responses in vivo to microbial antigens.

with hexamethylmelamine and cyclophosphamide, leads to normal responses, as shown in group C. Secondary antibody responses, elicited at the same time, were unaffected.

The drug regimens induced a transient decrease not only of the number of granulocytes, but—especially in group C (Hexa CAF)—also of the number of lymphocytes. This may well be due to the action of cyclophosphamide as demonstrated in our previous study [7].

The proliferative capacity of lymphocytes *in vitro* showed a moderate but only transient decrease in groups A and B (Chap-5), although the percentage of T lymphocytes was not altered. This indicates a direct effect of these drugs on the proliferative capacity of T lymphocytes. An effect on the antigen-presenting function of monocytes seems less likely, because the responses to non-specific mitogens (ALS, PHA, ConA, PWM) which are not strictly dependent on the presence of monocytes, were at least equally affected as the responses to specific antigens. The depressed proliferative capacity of lymphocytes in group A may be caused by *cis*platin. Khan and Hill [8] found a diminished proliferative capacity to phytohaemagglutinin (PHA) in 5 patients after administration of 1–4 mg *cis*platin/kg. However,

adriamycin, given in doses higher than used in the present study, did not affect cellular immune responses *in vitro* [3]. The decrease of proliferative capacity in group B may also be caused by *cis*platin, because this drug remains detectable in the tissues for at least 4 months after administration [13]. An effect of hexamethylmalamine and/or cyclophosphamide on this function may also be responsible.

The capacity to generate CTL was unaffected after a 7-day course of hexamethylmelamine and cyclophosphamide (group B), but decreased after treatment with these drugs for 14 days (group C). This decrease was reversible. At the time of diminished CTL reactivity, the proliferative capacity in the mixed lymphocyte culture (MLC) was normal. Therefore, the depression of generation of CTL might be due to an effect of these drugs on the number and/or function of cytotoxic T-cell precursors. This effect may be due to the action of hexamethylmelamine, because in our previous study we have shown that cyclophosphamide does not affect the development of CTL [7]. An additional effect of 5 FU and/or MTX cannot be excluded. However, this is less likely because of the short half-time of these drugs and the relative large interval between the last administration of these drugs and the collection of blood for the CML test (7 days).

The drug regimens studied had no effect on K- and NK-cell activity. This finding contrasts to that of Hersh *et al.* [11], who found a decrease of K-cell activity as tested against chicken erythrocytes in patients who received a combination chemotherapy including adriamycin, cyclophosphamide and *cis*platin. The difference in results may be due to the difference in assay system: in our study, mouse-mastocytoma cells were used as targets. In addition, the different drug regimens used and the differences in timing of study may have an effect.

The primary cellular immune response *in vivo* seems only minimally depressed. Obviously, in these patients any depressed response might be caused by either the malignancy [23] or the cytotoxic drug regimen. Due to the lack of a non-treated control group, these two possibilities cannot be discriminated. The drug treatment appears to have only marginal influence, if any at all.

No definite conclusion can be drawn regarding the influence of a particular chemotherapy scheme on skin reactivity to anamnestic antigens. The low reactivity of lymphocytes after stimulation with the same antigens *in vitro*, observed before initiation of therapy with cytotoxic drugs, suggests that either insufficient sensitization to these antigens had occurred or that the malignant process is responsible. It seems unlikely that chemotherapy would affect memory-cell function, because secondary humoral responses are not affected. A diminished expression of delayed-type hypersensitivity *in vivo*, due to an anti-inflammatory effect of the drug regimen seems also less likely, because of the presence of skin reactivity to DNCB.

In conclusion, the present study shows only a moderate influence of treatment with Chap-5 or Hexa CAF on the immunocompetence of patients with advanced ovarian carcinoma, studied during the first cycle of chemotherapy. Moreover, the observed effects on cell counts, proliferative capacity and cytotoxic function of the lymphocytes were rapidly reversible. During the period of this study, our patients showed an incidence and severity of infections which were not different from those observed in other patients in the same hospital.

Further studies are needed regarding the effects of repeated cycles of chemotherapy on the immune system.

Acknowledgements—We are grateful to Mrs. E. A. M. Hamersma-van der Linden for her contribution in collecting the patients. We thank Ms S. L. Yong, Ms M. Hansen and Ms F. de Wilde for technical assistance. Dr. A. M. Hagenaars and Dr. G. van Steenis (Rijks Instituut voor de Volksgezondheid, Bilthoven, The Netherlands) performed the determinations of antibody levels against diphtheria toxoid and polio virus. The antigen haemocyanin was a gift from Prof. Dr. T. H. The (Department of Internal Medicine, Division of Clinical Immunology, University Hospital, Groningen, The Netherlands). We are also grateful to Dr. C. J. M. Melief, Dr. T. A. Out, Dr. R. S. Weening and Dr. W. P. Zeijlemaker for critical reading of the manuscript.

REFERENCES

1. MITCHELL MS, WADE ME, DECONTI RC, BERTINO JR, CALABRESI P. Immunosuppressive effects of cytosine-arabinoside and methotrexate in man. *Ann Intern Med* 1969, **70**, 535–547.
2. HARRIS J, SENGAR D, STEWART TH, HYSLOP D. The effect of immunosuppressive chemotherapy on immune function in patients with malignant disease. *Cancer* 1976, **37**, 1058–1069.
3. ROTH JA, EILBER FR, MORTON DL. Effect of adriamycin and high-dose methotrexate chemotherapy on *in-vivo* and *in-vitro* cell-mediated immunity in cancer patients. *Cancer* 1978, **41**, 814–819.

4. WEBSTER DJT, RICHARDSON G, BAUM M, PRIESTMAN T, HUGHES LE. Effect of treatment on the immunological status of women with advanced breast cancer. *Br J Cancer* 1979, **39**, 676–680.
5. ALEXOPOULOS CG, WILTSHAW E. Immunological monitoring during chemotherapy for advanced Hodgkin's disease. *Cancer* 1978, **42**, 2631–2640.
6. STRENDER LE, BLOMGREN H, PETRINI B *et al.* Immunologic monitoring in breast cancer patients receiving postoperative adjuvant chemotherapy. *Cancer* 1981, **48**, 1996–2002.
7. TEN BERGE RJM, VAN WALBEEK HK, SCHELLEKENS PTHA. Evaluation of the immunosuppressive effects of cyclophosphamide in patients with multiple sclerosis. *Clin Exp Immunol* 1982, **50**, 495–502.
8. KHAN A, HILL JM. Suppression of lymphocyte blastogenesis in man following *cis*-platinous diamminodichloride administration (37015). *Proc Soc Exp Biol Med* 1973, **142**, 324–326.
9. KLEINERMAN ES, ZWELLING LA, HOWSER D *et al.* Defective monocyte killing in patients with malignancies and restoration of function during chemotherapy. *Lancet* 1980, **ii**, 1102–1105.
10. KLEINERMAN ES, ZWELLING LA, MUCHMORE AV. Enhancement of naturally occurring human spontaneous monocyte-mediated cytotoxicity by *cis*-diammine dichloro platinum (II). *Cancer Res* 1980, **40**, 3099–3102.
11. HERSH EM, MURPHY SG, GUTTERMAN JU *et al.* Antibody-dependent cell-mediated cytotoxicity in human cancer: characterization of patient leukocyte activity and treatment effects. *Cancer* 1982, **49**, 251–260.
12. KLEINERMAN ES, ZWELLING LA. The effect of *cis*-diammine dichloro platinum (II) on immune function *in vitro* and *in vivo* (Review). *Cancer Immunol Immunother* 1982, **12**, 191–196.
13. ROZENCWEIG M, VON HOFF DD, SLAVIK M, MUGGIA FM. *Cis*-diammine dichloroplatinum (II). A new anticancer drug. *Ann Intern Med* 1977, **86**, 803–812.
14. EDITORIAL. *Cis*platin. *Lancet* 1982, **1**, 374–375.
15. DU BOIS MJGJ, SCHELLEKENS PTHA, DE WIT JJFM, EIJSVOOGEL VP. *In-vitro* reactivity of human lymphocytes after cryopreservation using a programmed cooling device. *Scand J Immunol* 1976, **5** (Suppl.), 17–22.
16. VAN OERS MHJ, PINKSTER J, ZEIJLEMAKER WP. Cooperative effects in mitogen- and antigen-induced responses of human peripheral blood lymphocyte subpopulations. *Intern Archs Allergy Appl Immunol* 1979, **58**, 53–66.
17. LEGUIT P, MEINESZ A, HUISMAN L, EIJSVOOGEL VP. The use of an antigen cocktail in the lymphocyte transformation test. *Clin Exp Immunol* 1973, **14**, 149–152.
18. ZEIJLEMAKER WP, VAN OERS MHJ, EIJSVOOGEL VP. Human lymphocyte subpopulations involved in MLC and CML. *Scand J Immunol* 1976, **5** (Suppl.), 143–156.
19. ZEIJLEMAKER WP, ROOS MTHL, SCHELLEKENS PTHA, EIJSVOOGEL VP. Antibody-dependent human lymphocytotoxicity: a micro-assay system. *Eur J Immunol* 1975, **5**, 579–584.
20. ORTALDO JR, OLDHAM RK, CANNON GC, HERBERMAN RB. Specificity of natural cytotoxic reactivity of normal human lymphocytes against a myeloid leukemia cell line. *J Natl Cancer Inst* 1977, **59**, 77–82.
21. TEN BERGE RJM, SCHELLEKENS PTHA, SURACHNO S, THE TH, TEN VEEN JH, WILMINK JM. The influence of therapy with azathioprine and prednisone on the immune system of kidney-transplant recipients. *Clin Immunol Immunopathol* 1981, **21**, 20–32.
22. KHAN A, HILL JM. Immunosuppression with *cis*-platinum (II) diamminodichloride: effect on antibody plaque-forming spleen cells. *Infect Immun* 1971, **4**, 320–321.
23. WANEBO HJ. Immunologic testing as a guide to cancer management. *Surg Clin N Am* 1979, **39**, 323–347.