Clin. exp. Immunol. (1980) **42**, 41–49.

Immune status in Crohn's disease

V. DECREASED *IN VITRO* NATURAL KILLER CELL ACTIVITY IN PERIPHERAL BLOOD*

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(*Accepted for publication 18 April 1980*)

SUMMARY

To identify and measure the spontaneous cell-mediated cytotoxicity of natural killer cells, we used the lysis of an established lymphoblastoid cell line as target in a 4-hr ^{51}Cr-release assay. Mononuclear cell suspensions of the peripheral blood of thirty-four patients with Crohn's disease (group CD), eleven patients with inflammatory bowel disease other than CD or ulcerative colitis (group D) and forty-five healthy subjects matched for both age and sex with the patients were studied. Depletion of phagocytic, plastic-adherent cells ('purified suspensions') led to a significant increase of the natural killer cell activity as compared with unseparated suspensions. This was seen to occur in all groups. In CD patients the natural killer cell activity was significantly below normal levels in both unseparated and 'purified' suspensions. This was independent of disease duration. In 'purified' suspensions the natural killer cell activity was inversely related to the disease activity. In group D the natural cytotoxicity was significantly lower in unseparated suspensions than that in healthy controls. In 'purified' suspensions it was still slightly lower than in healthy controls.

INTRODUCTION

In man, lymphocytes exert cytotoxic activity against a variety of target cells *in vitro* without apparent previous sensitization (Takasugi, Mickey & Terasaki, 1973). The lymphoid cells mediating this spontaneous or natural cell-mediated cytotoxicity have been termed 'natural killer' (NK) cells. Their nature and mode of action have not been satisfactorily resolved; however, present evidence indicates that the NK cells comprise apparently two types of cell. One of these is a non-adherent, non-phagocytic, surface membrane immunoglobulin-negative ($SmIg^-$), Fcγ receptor-positive ($FcRIgG^+$) lymphoid cell (De Vries, Cornain & Rümke, 1974; Hersey *et al.*, 1975; West *et al.*, 1977; Bolhuis *et al.*, 1978. Most of these have putative T cell markers (Hersey *et al.*, 1975; West *et al.*, 1977; Bolhuis *et al.*, 1978). The other population represents an E-rosetting subset which lacks detectable receptors for Fcγ (Bolhuis *et al.*, 1978; Eremin *et al.*, 1978).

Studying the immune status in Crohn's disease (CD) we were particularly interested in the functional state of the NK cells in these patients for the following reasons. Firstly, on the one hand in CD virus-like agents have been found (Gitnick, Arthur & Slibata, 1976; Whorwell *et al.*, 1977), and on the other hand NK cells may play a role in protection and recovery from viral infection

* Parts of this work were presented at the Annual Meeting of the German Society of Immunology, 1979 (Auer & Ziemer, 1979).

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0099-9104/80/1000-0041$02.00

(Lemon, Hutt & Huang, 1977; Härfast, Andersson & Perlmann, 1978; Trinchieri & Santoli, 1978). Secondly, in CD a slight, though statistically significant, increased propensity to malignancy has been shown (Fielding *et al.*, 1972; Weedon *et al.*, 1973; Gyde *et al.*, 1979). In this regard it has been suggested that natural cell-mediated immunity might be linked to immune surveillance and *in vivo* resistance against tumour growth (Zarling, Nowinski & Bach, 1975; Haller *et al.*, 1977; Takasugi, Ramseier & Takasugi, 1977; Jondal, Spina & Targan, 1978). Therefore we investigated the NK cell activity in the peripheral blood (PB) in patients with CD and in simultaneously tested sex- and age-matched normals. To differentiate between possible alterations of the NK cell activity being primary (thus possibly predisposing to the disease) or secondary, a group of patients with inflammatory bowel disease other than CD or ulcerative colitis (group D) was studied. In addition, the group of CD patients was made up of two subgroups. One subgroup (CD1) consisted of a selected consecutive series of newly diagnosed patients who had short-standing disease and who had not received any specific therapy ('virgin Crohn's'). The other subgroup (CD2) were patients with long-standing disease, who had received drug treatment. Using this design we have recently been able to show that the numerical (Auer *et al.*, 1978b, 1979) and function (Auer, Buschmann & Ziemer, 1978a; Auer & Ziemer, 1980) alterations and deficiencies of some immune parameters in CD are not pre-existing, but are secondary to the disease.

MATERIALS AND METHODS

Patients and controls

Patients with CD (group CD). Thirty-four patients with CD were studied for NK cell activity. Group CD consisted of the two subgroups CD1 and CD2. Group CD1 was a selected consecutive series of sixteen newly diagnosed CD patients who had not received any drugs for their disease. Most of these had short-standing disease ('virgin Crohn's'; mean [$\bar{x}$] disease duration $\pm$ standard error [s.e.m.]: $24{\cdot}8 \pm 6{\cdot}7$ months; age [$\bar{x} \pm$ s.e.m.]: $30{\cdot}7 \pm 3{\cdot}5$ years). In group CD2 eighteen CD patients were collected (age [$\bar{x} \pm$ s.e.m.]: $28{\cdot}1 \pm 1{\cdot}8$ years) who had long-standing disease ($\bar{x} +$ s.e.m.: $59{\cdot}7 \pm 8{\cdot}0$ months) and who had been previously drug-treated with salicylazosulphapyridine (SASP, $n = 16$), steroids ($n = 13$) or azathioprine (AZA; $n = 1$). At the time of testing all patients had been off SASP, steroids and AZA for at least 3 weeks.

Disease activity was determined according to the Crohn's disease activity index (CDAI; Best *et al.*, 1976). In group CD1 eight patients had inactive mild disease (CDAI $\leqslant 150$), and eight a highly active disease (CDAI > 150); in group CD2 eight were inactive and ten highly active. The site of disease was comparable in groups CD1 and CD2, the disease being restricted to the small bowel in five and eight patients respectively, to both the small bowel and the colon in nine patients in each group and to the colon alone in two and one patients respectively.

Diseased controls (group D; age [$\bar{x} \pm$ s.e.m.]: 36 ± 17 years). The eleven subjects with inflammatory bowel disease other than ulcerative colitis or CD suffered from Salmonella gastroenteritis ($n = 5$), non-specific gastroenteritis ($n = 3$), diverticulitis colic ($n = 3$) and bacterial overgrowth ($n = 1$).

Normal controls (N group). Because of the reported dependency of the NK cell activity on both sex and age (Santoli *et al.*, 1976), for each patient one normal control subject was matched for both parameters and was bled at the same time of day and tested simultaneously with the corresponding CD or D patient. Thus forty-five healthy subjects were tested. These composed the normal control groups NCD1, NCD2, NCD and ND.

Isolation and 'purification' of peripheral blood (PB) mononuclear cells

Two methods were used to obtain effector cells. In the first method (method 1) effector cells from heparinized PB (10 u heparin/ml) were isolated by means of a Ficoll density gradient (Böyum, 1968) as outlined by Auer *et al.* (1978b). These cell suspensions were kept in RPMI 1640 (GIBCO) supplemented with glutamine (2 mmol/l), penicillin (50 u/ml) and streptomycin (100 μg/ml), termed 'culture medium'. In the other method (method 2) the PB cells were first depleted of phagocytic cells

by preincubation with carbonyl iron containing Lymphocyte Separating Reagent (Technicon) as described previously (Auer *et al.*, 1979) and thereafter subjected to Ficoll density gradient centrifugation. These mononuclear cell suspensions were then depleted of plastic-adherent cells as described by Auer & Ziemer (1980). No ammonium chloride buffer was used for lysis of the red blood cells since this abrogates NK cell activity.

Cell viability in the various suspensions was at least 97% as evaluated in the trypan blue exclusion test.

NK cell activity

NK cell activity was demonstrated in U-bottomed microtitre plates (Greiner Plastic) by a ^{51}Cr-release assay with an established lymphoblastoid cell line (LIK) as target as described by Trinchieri *et al.* (1973) and modified as follows.

Equal volumes (0·1 ml) of the target lymphoblasts (20×10^6 viable cells/ml) and $Na_2{}^{51}CrO_4$ (spec. act. 200–500 Ci/mmol Na_2CrO_4 in 0·9% NaCl; NEN) were incubated for 20 min at 37°C in 5% CO_2/95% air. After washing three times in phosphate-buffered saline supplemented with foetal calf serum (FCS) the target lymphoblasts were resuspended in RPMI 1640 culture medium at a concentration of $3{\cdot}3 \times 10^5$.

Effector lymphocytes in both unseparated cell suspensions and cell suspensions depleted of both plastic-adherent and phagocytic cells were resuspended at 1×10^7 viable cells/ml in RPMI 1640 culture medium. Dilutions of these containing 8, 4 and 2×10^6 cells/ml were also made.

The cytotoxic test was performed in triplicate cultures. Each well was given both 1×10^4 target lymphoblasts in 0·03 ml and 0·03 ml of heat-inactivated (56°C, 30 min) FCS. 0·1 ml effector cells in concentrations as described above were added to each of the spontaneous cell-mediated cytotoxicity (SCMC) wells, while the total release wells (T) received 0·1 ml of zaponin (10% in RPMI 1640; Coulter Electronics) and the spontaneous release wells (S) 0·1 ml of RPMI 1640 supplemented as described above. The test was incubated for 4 hr at 37°C. Thereafter, the microtitre plates were centrifuged at 4°C at 1,000 ***g*** for 5 min. Supernatant (0·1 ml) was removed from each well and counted for ^{51}Cr radioactivity using a Berthold Gammascint BF 5300.

The NK cell activity was evaluated both by means of the percentage specific ^{51}Cr-release (R) and by determination of absolute number of cells needed for a specific release of 10% (SC10) in the given time of incubation. The percentage specific ^{51}Cr-release (R) was calculated by:

$$R = \frac{\text{SCMC (c.p.m.)} - \text{S (c.p.m.)}}{\text{T (c.p.m.)} - \text{S (c.p.m.)}} \times 100.$$

The SC10, which is inversely related to the specific release, was calculated by a computer program (see Statistics) using the per cent specific release (R) of the effector cell dose–response curves. If an individual failed to exhibit a 10% specific lysis in the NK cell activity even at the highest (100:1) or lowest (20:1) effector/target cell ratios the computer was programmed to assume an arbitrary number of 2×10^6 and $0{\cdot}05 \times 10^6$ cells respectively as SC10 for computation.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

The ADCC activity was determined in a ^{51}Cr-release assay as described in a previous report using the same established lymphoblastoid cell line (LIK) as target (Auer & Ziemer, 1980).

Pretreatment of effector cells with CD sera or rabbit IgG

Effector cells (0·03 ml; $0{\cdot}4 \times 10^6$ cells/well) were preincubated at 4°C for 30 min with either 50 μl of twenty-three heat-inactivated undiluted sera of twenty-two different CD patients for evaluation of the influence of CD sera on the NK cell activity; or with 50 μl of heat-inactivated FCS for establishing the NK cell activity of the untreated control; or with 50 μl of heat-aggregated (63°C, 15 min) rabbit IgG (R-IgG-agg; Dickler, 1974) containing 500 μg of protein. The same effector cells were simultaneously studied in the ADCC.

Leucocyte populations and lymphocyte subsets

Absolute lymphocyte counts were determined as described previously (Auer *et al.*, 1978b). The relative proportions of T (E-rosetting), B (surface membrane immunoglobulin-bearing) and null-lymphocyte subsets were evaluated in leucocyte suspensions depleted of phagocytic cells as described by Auer *et al.* (1979).

Statistics

Results are given as mean ($\bar{x}$) $\pm$ 1 standard deviation (s.d.) unless otherwise stated. A TR 440 computer (Institute for Applied Mathematics, University of Würzburg) was used for statistical evaluation of the data obtained. Wilcoxon's matched-pair signed rank test (WMPSR) was used to evaluate the significance of difference between the various patients (CD and D patients respectively) and the corresponding, simultaneously tested normal controls, while Wilcoxon's two-sample rank tests (*U*-test) were applied for the other comparisons. The significance of correlations was tested by Spearman's rank correlation test (SR). All tests were two-tailed.

RESULTS

Unseparated leucocyte suspensions

NK cell activity, expressed as per cent specific ^{51}Cr-release from the lymphoblastoid target cell, was significantly lower in the thirty-four patients with CD than in the corresponding normal controls (Fig. 1). Very similar data were obtained in groups CD1 and CD2. Group D also showed a significantly lower NK cell activity than ND (Fig. 1). The NK cell activities in groups CD and D were comparable.

'Purified' leucocyte suspensions

Depletion of the leucocyte suspensions of both phagocytic and plastic adherent cells resulted in a significant increase of the NK cell activity in group CD (Fig. 1), groups CD1 and CD2 alike (significance of increase in group CD: at 0·4 and 1·0 $\times$ 10^6 effector cells/well = $P < 0{\cdot}05$; at 0·8 $\times$ 10^6 effector cells/well = $P < 0{\cdot}005$). However, even after this treatment the NK cell activity was still significantly lower in CD patients, both in CD1 and CD2, than in corresponding normal controls ($n = 13$; Fig. 1) since the normal control group showed a significant increase in the NK cell activity after 'purification' (significance of increase in group NCD = $P < 0{\cdot}01$ for all effector lymphocyte/-target cell ratios). A similar increase was also seen in both groups D and ND ($P = 0{\cdot}05$ for all effector/target cell ratios). In the 'purified' suspension of group D the NK cell activity was still slightly lower than in normals (Fig. 1).

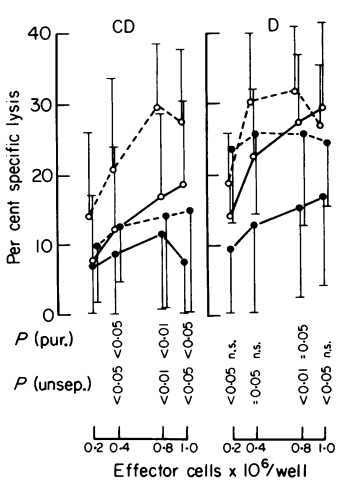

Fig. 1. NK cell activity (per cent specific lysis) of unseparated (solid lines) and 'purified' (broken lines) leucocyte suspensions of patients as compared with normal controls. (●) Patient groups as indicated; (○) corresponding normal controls as indicated.

Effect of sera of CD patient on the NK cell activity

To gain information regarding the influence of sera of CD patients on the NK and K cell activity, effector cells ($0{\cdot}4 \times 10^6$/well) of two healthy donors were preincubated with a total of twenty-three sera from twenty-two CD patients. Because of the claim that NK cell activity might in effect represent antibody-dependent cell-mediated (ADCC) cytotoxicity due to natural antibodies (Akira & Takasugi, 1977), the effector cells were also preincubated with heat-aggregated rabbit IgG (R-IgG-agg). Fig. 2 summarizes the data on the results of this treatment of the effector cells. While

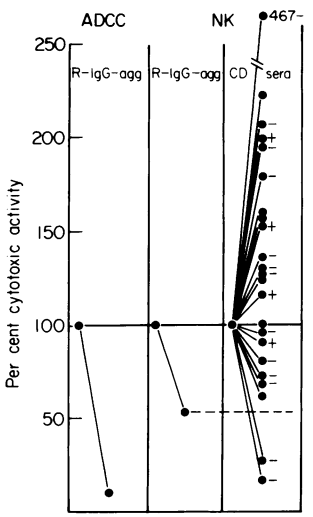

Fig. 2. Influence of CD sera and heat-aggregated rabbit IgG (R-IgG-agg) on the NK cell activity of normal effector cells. For comparison the influence of R-IgG-agg on the ADCC activity is also given. The change in the per cent cytotoxic activity relates to the per cent specific cytotoxicity in the NK cell assay and ADCC assay, respectively, of the control cultures (incubated with FCS), which were taken as 100% cytotoxic activity. CD sera positive or negative for lymphocytotoxic antibodies against the target are indicated with + and − respectively.

R-IgG-agg led to approximately 90% inhibition of the ADCC, the same R-IgG-agg-pretreated effector populations still exhibited more than one-half of the original NK activity. A strong inhibitory effect on NK cells was seen in two of the twenty-three CD sera; however, most of the CD sera enhanced the NK cell activity. Seventeen sera, including ten of the fourteen sera with enhancing activity, were tested for lymphocytotoxic antibodies against the target cells at 15 and 22°C. As indicated in Fig. 3, only four sera were positive.

Relationship with disease parameters

In group CD as a whole the per cent specific release due to NK cells was below normal levels. The depression was not significantly influenced by disease activity in either unseparated or 'purified' suspensions (Fig. 3). However, with 'purified' suspensions, there was a trend ($0{\cdot}1 \geqslant P > 0{\cdot}05$) for patients with active CD (CDAI > 150) to have a lower NK activity than patients with inactive disease (Fig. 3).

Thus, using 'purified' lymphocyte suspensions from CD patients with active disease (CDAI > 150) significantly more effector cells were needed to obtain 10% specific ^{51}Cr-release (SC10) than in those with quiescent or mild disease (CDAI $\ll$ 150; $P < 0{\cdot}05$).

There was a highly significant trend towards a lower NK cell activity with increasing disease duration in all CD groups. However, when the natural cytotoxicity of the normal controls was correlated with the disease duration of the corresponding sex- and age-matched CD patients, the same correlation was observed. This points towards an incidental nature of this statistically significant finding in CD.

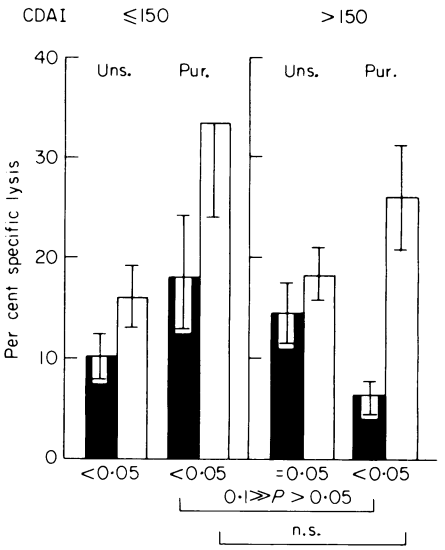

Fig. 3. NK cytotoxicity (per cent specific lysis) in CD patients (■) and normal controls (□). The CD patients are separated according to inactive (CDAI ≤ 150) and active (CDAI > 150) disease. Uns. = unseparated cell suspensions; pur. = 'purified' cell suspensions.

Dissociation between NK cell cytotoxicity and ADCC activity in CD

In the 'purified' suspensions of thirteen patients with CD and their corresponding controls both NK cell and ADCC activity were simultaneously determined. As is evident from Fig. 4, the NK cell activity was decreased (less than 70% of the corresponding normal control) not only in those CD patients who showed impaired ADCC activity, but also in 63·5% (5/8) of the CD patients with normal ADCC.

Correlation with white cell differential count and leucocyte subsets

In 'purified' suspensions from CD patients the numbers of cells needed to obtain 10% specific release (SC10) was higher the more juvenile neutrophils were present in the PB ($SR + 0{\cdot}5510$, $n = 13$, $P < 0{\cdot}05$). There was no significant consistent correlation of the NK cell activity with T, B or Null cells.

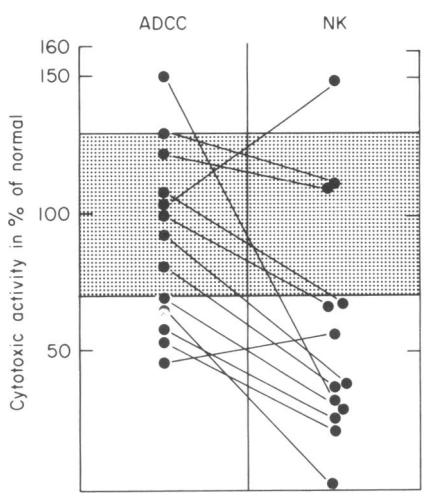

Fig. 4. Dissociation in the activity of the ADCC and NK cell cytotoxicity in individual CD patients. The range of ±30% of the cytotoxic activity of the corresponding normal controls is arbitrarily considered as normal.

DISCUSSION

Only three out of ninety subjects tested in this study failed to lyse the lymphoblastoid target. One of them was a CD patient with the HLA-A3, -B7 haplotype (not shown in the data). A general lack of spontaneous lymphocytotoxic activity in normal subjects carrying this HLA haplotype has been reported (Petranyi *et al.*, 1974; Santoli *et al.*, 1976).

The observation in this study that depletion of phagocytic plastic-adherent cells led to augmentation of NK cell cytotoxicity both in patients and controls agrees with that of West *et al.* (1977), who found a similar pattern in normals.

The most important finding in the present work was an impairment of the NK cell activity in both unseparated and 'purified' suspensions of both active (CDAI > 150) and inactive (CDAI ≤ 150) patients with CD. The mode and nature of this impairment are unclear at present.

Inhibition of NK cell activity by prostaglandins has been reported (Droller, Schneider & Perlmann, 1978). In the present study NK cell activity could be suppressed by immune complexes to a certain extent. Both of these factors might be increased in CD. However, in view of our experiments evaluating the influence of CD sera on the NK cell activity of normals, such serum factors do not seem to be likely as major causes of the impaired NK cell activity. These experiments showed an inhibitory influence (less than 70% cytotoxic activity) in less than 20% of the CD sera, while the majority of CD sera lead to an increase of the NK cell activity, similar to the effect of sera of healthy people (Györffy, Petranyi & Benczur, 1978). Only three out of ten CD sera with such enhancing activity showed lymphocytotoxins against the target cells. However, since the sensitivity by which ADCC detects antibody reactivity is 100- to 1,000-fold greater than the sensitivity of the complement-dependent system (Dickmeiss & Nielsen, 1975), a possible role of such antibodies for this enhancement, which would then reflect an ADCC, cannot entirely be excluded.

More recently, it has been suggested that NK cell activity is regulated by levels of interferon found *in vivo* (Trinchieri & Santoli, 1978). Thus, depressed interferon production as reported in chronic hepatitis in children (Tolentino *et al.*, 1975) or multiple sclerosis (Neighbour & Bloom, 1979) or decreased susceptibility of NK cells to interferon might play a role in this impairment of the NK cell activity in CD. However, sequestration of NK cells in the gut would be another possibility, although the presence of NK cells in the gut is a matter of debate (Arnaud-Battandier *et al.*, 1978; MacDermott *et al.*, 1979).

This study revealed a suppressive influence of the disease activity on the NK cell activity in 'purified' suspensions in CD. Similarly, as shown in a previous report (Auer & Ziemer, 1980), the ADCC was decreased in active CD patients (CDAI > 150) of the same group. However, while inactive (CDAI ≤ 150) CD patients had a virtually normal ADCC activity in 'purified' suspensions (Auer & Ziemer, 1980), the NK cell activity was significantly decreased in CD patients with mild disease. This dissociation in the activity of the ADCC and NK cell cytotoxicity in CD is in agreement with a very recent observation by Jones, Gitnick & Targan (1979). These authors reported that CD tissue filtrates or tissue culture fluid from cell lines inoculated with CD filtrate produced a strong inhibition of NK cell activity while there was no modulation of the ADCC. This dissociation is consistent with the hypothesis that, at least in part, ADCC and NK cell cytotoxicity are mediated by separate mechanisms. This view receives further support from the observation that in the assay where aggregated rabbit IgG, which has been shown to block the FcR (Dickler, 1974), was added to cultures it inhibited ADCC activity by more than 90%, while the NK cell activity was reduced by less than one-half. This is similar to the inability of others to inhibit completely NK cell activity by immune complexes (Bolhuis *et al.*, 1978).

There was an inverse relationship between NK cell activity and the proportion of juveniles, which is increased in active disease (Auer *et al.*, 1978b). Juveniles might directly and negatively influence NK activity and thus in part be responsible for this CDAI-dependent additional decrease of the NK cell activity. Alternatively, both changes might relate independently to the disease activity.

The NK cell activity is equally decreased in 'virgin Crohn's' with short disease duration (group CD1) and in group CD2. This impairment, therefore, appears to be independent of disease duration

and is present already at an early stage of the disease, when certain T and B cell parameters are unaltered (Auer *et al.*, 1978a, 1978b, 1979). There was also a strong tendency to an impairment of the NK cell activity in the control patients with common infectious and inflammatory bowel diseases (group D). Consequently the decrease in the NK cell activity even in 'virgin Crohn's' (group CD1) might have to be considered as due to the disease rather than as a pre-existing, possibly predisposing, factor for CD.

However, while NK cell impairment is transient in D patients, it seems to be chronic in CD, and even pronounced in active disease. Whether this chronic impairment may relate to the appearance of virus-like agents (Gitnick *et al.*, 1976; Whorwell *et al.*, 1977) and/or the slightly, though significantly increased propensity to high-grade malignancy in CD patients (Fielding *et al.*, 1972; Weedon *et al.*, 1973; Gyde *et al.*, 1979) remains to be established.

The authors gratefully acknowledge the expert technical assistence of Miss R. Hilbert and Mrs Ch. Rompf-Buschmann, the advice in the statistical work by Dr Vogt (Institute of Statistics, University of Würzburg) and the determination of the lymphocytotoxic antibodies by Dr Wiebecke (Dept of Surgery, University of Würzburg).

The study has been supported by DFG Au 35/4, Au 35/5 and Au 35/6 from Deutsche Forschungsgemeinschaft.

REFERENCES

AKIRA, D. & TAKASUGI, M. (1977) Loss of specific natural cell mediated cytotoxicity with absorption of natural antibodies from serum. *Int. J. Cancer*, **19**, 747.

ARNAUD-BATTANDIER, F., BUNDY, B.M., O'NEILL, M., BIENENSTOCK, J. & NELSON, D.L. (1978) Cytotoxic activities of gut mucosae lymphoid cells in guinea pigs. *J. Immunol.* **121**, 1059.

AUER, I.O., BUSCHMANN, CH. & ZIEMER, E. (1978a) Immune status in Crohn's disease. II. Originally unimpaired primary cell mediated immunity *in vitro*. *Gut*, **19**, 618.

AUER, I.O., GÖTZ, S., ZIEMER, E., MALCHOW, H. & EHMS, H. (1979) Immune status in Crohn's disease. III. Peripheral blood B-lymphocytes, enumerated by means of $F(ab)_2$-antibody fragments, Null and T lymphocytes. *Gut*, **20**, 261.

AUER, I.O., WECHSLER, W., ZIEMER, E., MALCHOW, H. & SOMMER, H. (1978b) Immune status in Crohn's disease. I. Leukocyte and lymphocyte subpopulations in peripheral blood. *Scand. J. Gastroenterol.* **13**, 561.

AUER, I.O. & ZIEMER, E. (1979) Natural killer cell activity and antibody dependent cell mediated cytotoxicity in Crohn's disease. *Immunobiology*, **156**, 227 (abstract 11.1).

AUER, I.O. & ZIEMER, E. (1980) Immune status in Crohn's disease. IV. *In vitro* antibody dependent cell mediated cytotoxicity in the peripheral blood. *Klin. Wochenschr.* **58**, 779.

BEST, W.R., BECKTEL, J.M., SINGLETON, J.W. & KERN, F. (1976) Development of a Crohn's disease activity index. National cooperative Crohn's disease study. *Gastroenterology*, **70**, 439.

BOLHUIS, R.L.H., SCHUIT, H.R.E., NOOGEN, A.M. & RONTELTAP, C.P. (1978) Characterisation of natural killer (NK) cells and killer (K) cells in human blood: discrimination between NK cell and K cell activities. *Eur. J. Immunol.* **8**, 731.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation by 1 *g*. *Scand. J. clin Lab. Invest.* **21** (Suppl. 97), 77.

DE VRIES, J.E., CORNAIN, E. & RÜMKE, PH. (1974) Cytotoxicity of non-T versus T-lymphocytes from melanoma patients and healthy donors on short- and longterm cultured melanoma cells. *Int. J. Cancer*, **14**, 427.

DICKLER, H.B. (1974) Studies of the human lymphocyte receptor for heat aggregated or antigen-complexed immunoglobulin. *J. exp. Med.* **140**, 508.

DICKMEISS, E. & NIELSEN, L.S. (1975) Antibody-dependent lymphocyte mediated cytotoxicity in an allogeneic human system. II. Evidence for HLA-specificity. *Scand. J. Immunol.* **3**, 199.

DROLLER, M.J., SCHNEIDER, M.U. & PERLMANN, P. (1978) A possible role of prostaglandins in the inhibition of natural and antibody dependent cell mediated cytotoxicity against tumour cells. *Cell. Immunol.* **39**, 165.

EREMIN, O., COOMBS, R.A., PLUMB, D. & ASHBY, J. (1978) Characterisation of the human natural killer (NK) cell in blood and lymphoid organs. *Int. J. Cancer*, **21**, 42.

FIELDING, J.F., PRIOR, P., WATERHOUSE, J.A. & COOKE, W.T. (1972) Malignancy in Crohn's disease. *Scand. J. Gastroenterol.* **7**, 3.

GITNICK, G.L., ARTHUR, M.H. & SLIBATA, J. (1976) Cultivation of viral agents from Crohn's disease. *Lancet*, **ii**, 215.

GYDE, S., MACCARTNEY, J., PRIOR, P., WATERHOUSE, J.A. & ALLEN, R.N. (1979) Cancer and Crohn's disease. *Autumn Meeting; Br. Soc. Gastroenterol.* **92** (abstract F 38).

GYÖRFFY, G.Y., PETRANYI, G.Y. & BENCZUR, M. (1978) The effect of human AB serum on the NK cell activity. *4th European Immunology Meeting, Budapest*, **72** (abstract).

HALLER, O., HANSSON, M., KIESSLING, R. & WIGZELL, H. (1977) Role of nonconventional natural killer cells in resistance against syngeneic tumour cells *in vivo*. *Nature*, **270**, 609.

HÄRFAST, B., ANDERSSON, T. & PERLMANN, P. (1978) Immunoglobulin independent natural cytotoxicity

of Fc-receptor-bearing human blood lymphocytes to mumps virus-infected target cells. *J. Immunol.* **121,** 75.

HERSEY, P., EDWARDS, A., EDWARDS, J., ADAMS, E., MILTON, G.W. & NELSON, D. (1975) Specificity of cell mediated cytotoxicity against human melanoma lines: evidence for 'non-specific' killing by activated T-cells. *Int. J. Cancer*, **16,** 173.

JONDAL, M., SPINA, C. & TARGAN, S. (1978) Human spontaneous killer cells selective for tumor-derived target cells. *Nature*, **272,** 62.

JONES, M.B., GITNICK, G. & TARGAN, S.R. (1979) Inhibition of spontaneous killer cell cytotoxicity by Crohn's disease tissue and cultures containing cytopathic agents. *Gastroenterol.* **76,** 1163 (abstract).

LEMON, S.M., HUTT, M. & HUANG, Y.T. (1977) Cytotoxicity of circulating leukocytes in cytomegalovirus mononucleosis. *Clin. Immunol. Immunopathol.* **i,** 513.

MACDERMOTT, R.P., JANKINS, K.M., FRANKLIN, G.D., WEINRIEB, I.J., NASH, G.S. & KODNER, I.J. (1979) Antibody dependent, spontaneous, and lectin induced cellular cytotoxicity by human intestinal lymphocytes. *Gastroenterology*, **76,** 1190 (abstract).

NEIGHBOUR, P.A. & BLOOM, B.R. (1979) Absence of virus-induced lymphocyte suppression and interferon production in multiple sclerosis. *Proc. Natl. Acad. Sci. USA*, **76,** 476.

PETRANYI, G.G., BENCZUR, M., ONODY, C.E. & HOLLAN, S.R. (1974) HL-A3,7 and lymphocyte cytotoxic activity. *Lancet*, **i,** 736.

SANTOLI, D., TRINCHIERI, G., ZMIJEWSKI, C.M. & KOPROWSKI, H. (1976) HLA-related control of spontaneous and antibody dependent cell mediated cytotoxic activity in humans. *J. Immunol.* **117,** 765.

TAKASUGI, M., MICKEY, M.R. & TERASAKI, P. (1973) Reactivity of lymphocytes from normal persons on cultured tumour cells. *Cancer Res.* **33,** 2898.

TAKASUGI, M., RAMSEIER, A. & TAKASUGI, J. (1977) Decline of natural non selective cell mediated cytotoxicity in patients with tumour progression. *Cancer Res.* **37,** 413.

TOLENTINO, P., DIANZANI, F., ZUCCA, M. & GIACCHINO, R. (1975) Decreased interferon response by lymphocytes from children with chronic hepatitis. *J. infect. Dis.* **132,** 459.

TRINCHIERI, G., DE MARCHI, M., MAYR, W., SAVI, M. & CEPELLINI, R. (1973) Lymphocyte antibody lymphocytolytic interaction (LALI) with special emphasis on HLA. *Transplant. Proc.* **5,** 1631.

TRINCHIERI, G. & SANTOLI, D. (1978) Antiviral activity induced by culturing lymphocytes with tumor-derived or virus transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J. exp. Med.* **147,** 1314.

WEEDON, D.D., SHORTER, R.G., ILSTRUP, D.M., HUIZENGA, K.A. & TAYLOR, W.F. (1973) Crohn's disease and Cancer. *N. Engl. J. Med.* **289,** 1099.

WEST, W.H., CANNON, G.B., KAY, D.H., BONNARD, G.D. & HERBERMANN, R.B. (1977) Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterisation of effector cells. *J. Immunol.* **118,** 355.

WHORWELL, P., PHILIPPS, C.A., BEEKEN, W.L., LITTLE, P.K. & ROESSNER, K.D. (1977) Isolation of reovirus-like agents from patients with Crohn's disease. *Lancet*, **i,** 1169.

ZARLING, J.M., NOWINSKI, R.C. & BACH, F.H. (1975) Lysis of leukemia cells by spleen cells of normal mice. *Proc. Natl. Acad. Sci. USA*, **72,** 2780.