Eur J Cancer, Vol. 29A, No. 3, pp. 474–475, 1993.
Printed in Great Britain
0964–1947/93 $6.00 + 0.00

Letters

Role of Interleukin-2 in Regulating Lymphocyte Activation and Recirculation

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TO UNDERSTAND better the immunological reactions primed by interleukin-2 (IL-2) in cancer patients we have investigated the biological effects of systemic rIL-2 on the circulating lymphocytes of cancer patients [1, 2]. We report here immunological tests, either functional or phenotypic, in 8 patients with advanced renal cell carcinoma treated with rIL-2 (18×10^6 U/m^2/day) by continuous intravenous administration for 5 consecutive days. Heparinised blood samples were drawn immediately before starting the first treatment cycle, 24 and 96 h after starting the rIL-2 infusion, and 2 days after the end of the infusion (168 h). Mononuclear cells were obtained by centrifugation over Ficoll gradient and resuspended in RPMI 1640 medium (Biochrom, Berlin, FRG). Phenotypic analysis was performed in a direct, double-staining, immunofluorescence assay. During lymphopenia, induced by the IL-2 administration, we observed a progressive decrease in the percentage of the CD3+CD4+ T-lymphocytes, while the CD3+CD8+ subset did not change significantly. Small CD4+ and CD8+ T cells are extracted by high endothelial venules with different efficiency (higher for the CD4+ than for the CD8+ subset) [3] and the rIL-2 infusion seems to emphasise this difference. The hypothesis that the decrease of the CD4+ T cells might be due to increased extravasation, is also sustained by concomitant reduced proliferation of the patients' peripheral blood mononuclear cells (PBMC) cultured for 3 days with different concentrations of phytohaemaglutinin (PHA) or concavalin A (ConA) (Sigma Chemical Co, St. Louis, Missouri). On stopping the rIL-2 infusion, when a rebound lymphocytosis is observed, the percentage of the CD4+ T-lymphocytes, and the capacity of PBMC to proliferate *in vitro* in response to lectins, return to baseline, but with a significant increase of CD4+ cells bearing activation markers (CD25 and DR) (Fig. 1). Since functionally different human CD4+ subsets, depending on their phenotypic expression of the CD45 isoforms, have been defined (CD45RA or naive and CD45RO or memory cells) [4, 5], we decided to monitor the variations of both subsets. During the rIL-2 infusion cycle the decrease of the CD4+ T-lymphocytes is sustained by a decrease in the percentage of the CD45RO subset, while the CD45RA subset increases. Conversely, 2 days after the end of the cycle the CD45RA subset falls and the CD45RO subset rebounds. The observation in sheep of a different recirculation

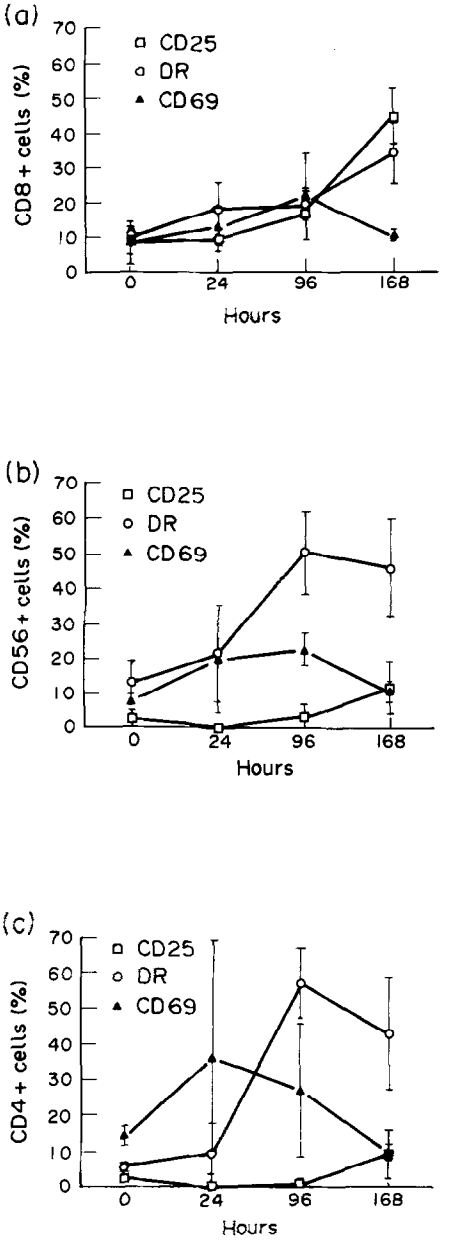

Fig. 1. Percentage of circulating (a) CD4+, (b) CD8+ and (c) CD56+ cells bearing surface activation markers during a cycle of rIL-2 administration.

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Revised 28 July 1992; accepted 6 Aug. 1992.

pathway of memory and naive CD4+ cells [6], and the capacity of the CD4+CD45RO subset to concentrate in the inflammation area [7] or to infiltrate subcutaneous metastases of human melanoma [8], further emphasise, in accordance with our results, the involvement of CD4+ T cells during *in vivo* IL-2 administration and their increased recirculation, in particular for the memory subset. Conversely, CD56+CD3− natural killer (NK) cells, after a slight and not significant decrease in the percentage 24 h after starting the IL-2 infusion, show a marked increase, peaking on the last day of the cycle. Even 2 days after stopping the infusion NK cells remain at a value higher than the baseline. It is also interesting to note the slight increase of circulating lymphocytes bearing the CD69 surface antigen, a molecule described as being acquired early after activation of T lymphocytes and NK cells [9, 10]. While the increase of the CD4+ and CD8+ T-lymphocyte subsets bearing the CD69 antigen is very slight and peaks the last day of the infusion cycle, the CD69+ NK cells increase early and markedly, peaking after 24 h of rIL-2 infusion (Fig. 1). The expression of this molecule precedes the expression of other activation molecules such as IL-2R and DR [10], suggesting an early involvement of circulating NK cells and of a smaller percentage of T-lymphocytes during *in vivo* rIL-2 administration.

In conclusion, our results strengthen previous observations of the involvement of NK cells and CD4+ T-lymphocytes, in particular with memory phenotype, in the immune modulation induced in cancer patients treated with systemic IL-2, and the role of this lymphokine in regulating lymphocyte recirculation.

1. Fortis C, Ferrero E, Biffi M, *et al.* Recombinant interleukin-2 and lymphokine-activated killer cells in renal cancer patients: II. Characterization of cells cultured *ex vivo* and their contribution to the *in vivo* immunomodulation. *Cancer Immunol Immunother* 1991, **33**, 128–132.
2. Fortis C, Ferrero E, Besana C, *et al.* Recombinant interleukin-2 and lymphokine-activated killer cells in renal cancer patients: I. Phenotypic and functional analysis of the peripheral blood mononuclear cells. *Cancer Immunol Immunother* 1990, **32**, 161–166.
3. Washington EA, Kimpton WG, Cahill RNP. CD4+ lymphocytes are extracted from blood by peripheral lymph nodes at different rates from other T cell subsets and B cells. *Eur J Immunol* 1988, **18**, 2093–2096.
4. Morimoto C, Letvin NL, Distaso JA, Aldrich WR, Schlossman SF. The isolation and characterization of the human suppressor inducer T cell subset. *J Immunol* 1985, **134**, 1508–1515.
5. Smith SH, Brown MH, Rowe D, Callard RE, Beverley PCL. Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UOIIL1. *Immunology* 1986, **58**, 63–70.
6. Mackay CR, Marston WL, Dudler L, Naive and Memory T cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 1990, **171**, 801–817.
7. Pitzalis C, Kingsley G, Haskard D, Panayi G. The preferential accumulation of helper-inducer T lymphocytes in inflammatory lesions: evidence for regulation by selective endothelial and homotypic adhesion. *Eur J Immunol* 1988, **18**, 1397–1404.
8. Cardi G, Mastrangelo MJ, Berd D. Depletion of T cells with the CD4+CD45R+ phenotype in lymphocytes that infiltrate subcutaneous metastases of human melanoma. *Cancer Res* 1989, **49**, 6562–6565.
9. Cosulich ME, Rubartelli A, Risso A, Cozzolino F, Bargellesi A. Functional characterization of an antigen involved in an early step of T-cell activation. *Proc Natl Acad Sci USA* 1987, **84**, 4205–4209.
10. Cebrià M, Yagüe E, Rincòn M, Lòpez-Botet M, de Landàzuri MO, Sànchez-Madrid F. Triggering of T cell proliferation through AIM, an activation inducer molecule expressed on activated human lymphocytes. *J Exp Med* 1988, **168**, 1621–1637.

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The Contribution of the Aminopyrine-breath-test in Metastatic Liver Disease

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THE 14C-aminopyrine-breath-test (BTA) is a well established functional liver test [1]. Surprisingly, there are no data in the literature reporting the use of the test in metastatic liver disease.

We here report our preliminary results, on the prognostic value of 14C-BTA in metastatic disease.

The test was performed after an overnight fast in the classical manner[1]. In 14 normal subjects [8 males, 6 females; average age mean (S.D.) 50 (13.5) years] the excretion rate was 5.2 (1.28)%. Patients with cancer but without liver metastases before or after chemotherapy showed a mean result of 4.4 (1.3)% [20 males, 14 females; average age 44 (16) years].

Patients without cancer, but with proven diffuse liver disease (5 males and 2 females; average age 56 (12) years) showed marked reduction [1.66 (0.66) %] and 14 patients with cancer without liver metastases but with known non-malignant hepatic disease [14 cases; 9 males, 5 females; average age 49 (8) years] also showed abnormal BTA values [1.9 (0.8)%].

19 patients with proven liver metastases were tested prior to chemotherapy. Of these 1 had a solitary metastasis, 15 multiple metastases and 3 had diffuse liver involvement. 3 patients with multiple metastases and all 3 with diffuse liver involvement had an abnormal (lower than 3%) BTA value. Of these 6 cases biochemical liver tests were only slightly disturbed (lowered PTT) in 4 patients. Nevertheless 4 patients died rapidly with advancing disease and signs of hepatic failure.

Of 24 patients with liver metastases who received several courses of chemotherapy, 9 had abnormal BTA values (lower than 2.5%). In 6/9 cases conventional liver function tests were only slight perturbed. Nevertheless the patients with low BTA values died rapidly with terminal hepatic failure and abnormal echographic and/or computed tomography findings.

In conclusion, in patients with liver metastases, a significantly lowered 14C-BTA value, either before, during or after chemotherapy, is a prognostic index even if concurrent biochemical liver tests are normal or only slightly disturbed.

1. Hepner GW, Vesell ES. Assessment of aminopyrine metabolism in man by breath analysis after oral administration of 14C-aminopyrine. (effects of phenobarbital, disulfiram and portal cirrhosis). *N Engl J Med* 1974, **291**, 1384–1388.

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Received 16 Apr. 1992; accepted 29 June 1992.