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Letter to the Editor

Evidence for the Non-reactive Nature of Lymphoid Elements in Serous Effusions from Patients with Cancer

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IT HAS BEEN suggested that the presence of T-cell components, which are characteristically seen in serous effusions from patients with non-haemopoietic malignancies [1], could be due to either an active host immune response [2] or alternatively to physical obstruction of central lymphatics by tumour or mediastinal lymphadenopathy [3]. In order to further evaluate the possible reactive nature of lymphoid populations, we have studied an additional 24 serous effusions (pleural fluid, $n = 20$; ascites, $n = 4$) and specifically assessed the expression of membrane determinants associated with T-cell 'activation'.

Serous effusions were obtained from patients with primary malignancies of the bronchus ($n = 2$), breast ($n = 13$), ovary ($n = 4$), prostate ($n = 1$) and parotid ($n = 1$); a further three patients were included whose tumour type had not been established at the time of analysis. Immunological studies for the demonstration of lymphocyte membrane determinants, performed in all cases within 12 h of aspiration, were achieved by indirect rosetting [4] and the observed percentages of positive cells corrected to exclude the influence of co-existing mesothelial cells and macrophages (mean observed 26.4%). Effusion T- and B-cell components were defined by monoclonal antibodies T11 (CD2) and HD37 (CD19) respectively and helper and suppressor T-cell subpopulations estimated using CD4 and CD8 monoclonal reagents. Monoclonal antibodies used to assess membrane changes associated with 'activation' included Ia (HLADr), IL-2 receptor (CD25) and PTS145 (CD26: kindly provided by Dr Ueda, Nagoya, Japan). In addition, studies were also undertaken to define, using monoclonal antibody Ki-67 against a nuclear proliferation antigen [5, 6], whether the lymphoid cells in these effusions were in resting (G0 and early G1) or active (late G1, G2 and S) stages of the cell cycle.

The total cell counts of the effusions examined ranged from 0.1–2.5 × 10^9/l (mean 0.74, median 0.75). The mean percentage of $CD2^+$ T-cells and $CD19^+$ B-cells were, after exclusion of mesothelial cells and histiocytes/macrophages, 91.6% (median 92.5, range 78–99%) and 7.4% (median 6.5, range 1–22) respectively. Although immunological studies with cancer-associated antibodies were not undertaken, morphological assessments did not suggest the presence of malignant cells in any of the effusions examined. Examination of activation markers on the effusion T-cell populations indicated that, in all cases studied ($n = 20$), the proportion of Ia^+ (after excluding the contribution of Ia^+ B-cells and histiocytes/macrophages) was <20%. Evaluation of the data for CD25 expression was more complicated in that variable proportions of histiocytes/macrophages also expressed this membrane determinant. However, when the cases with <80% lymphocytes and/or >10% histiocytes/macrophages were excluded from the analysis, it was found that the mean CD25 expression was 17.6% (median 13.5: $n = 16$) and that only three of these cases showed in excess of 30% CD25

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positive cells. For comparison, the CD26 T-cell activation antigen was found on a mean 33.9% of cells (median 24, range 1–88%) with 5/11 cases showing >30% positive cells. Thus, if positivity for the CD25 and CD26 antigens are arbitrarily defined as >30% positive cells, then of the 11 cases in which both determinants were examined the following phenotypic patterns could be shown; $CD25^-$ $CD26^-$ ($n = 6$), $CD25^-$ $CD26^+$ ($n = 2$) and $CD25^+$ $CD26^+$ ($n = 3$); no cases were found with an apparent $CD25^+$ $CD26^-$ phenotype. Although this suggested some correlation between the expression of these determinants, this was only just significant at the 5% level ($P = 0.046$: Spearman's coefficient).

The question of whether possibly reactive T-cell components were restricted to the localized enviroment of the serous effusion or whether this was a generalized clinical phenomenon within any given patient was additionally investigated by comparing the peripheral blood and serous effusion lymphocyte phenotypic patterns in eight of these cancer patients. Due to the presence of histiocytes/macrophages with CD4 expression (Fig. 1), CD4:CD8 ratios in serous effusions were not determined. However, paired statistical analyses showed no significant differences in relative B-cell ($CD19^+$) and T-suppressor ($CD8^+$) subpopulation distributions between the effusion and peripheral blood and although there was some difference in the proportions of $CD25^+$ cells ($P = 0.03$), this was due to lower numbers of effusion lymphocytes expressing this determinant. The conclusion that lymphocytes in serous effusions show little or no specific 'activation' was further supported by a study of Ki-67 expression in five cases. In these, the lymphoid populations were consistently $Ki\text{-}67^-$, in contrast to the non-lymphoid elements in which strong nuclear staining was often seen, and indicates that these lymphocytes were not in an 'active' stage of the cell cycle.

A variety of physical mechanisms are thought to contribute to the production of serous effusions in patients with malignant disease [7]. These include an increased rate of fluid and protein leakage into the serous cavity from abnormal capillaries and a reduction in the absorption rate secondary to the obstruction by tumour of capillaries and lymphatics within the visceral serous membranes. 'Central' lymphatic or venous obstruction (e.g. infiltration of hilar lymph nodes or superior vena caval obstruction) also contribute in some cases. Although the aetiology of serous effusions in patients with cancer may be multifactorial, the results of this current investigation suggest that the constituent lymphoid components in these effusions are not involved in an active host immunological response.

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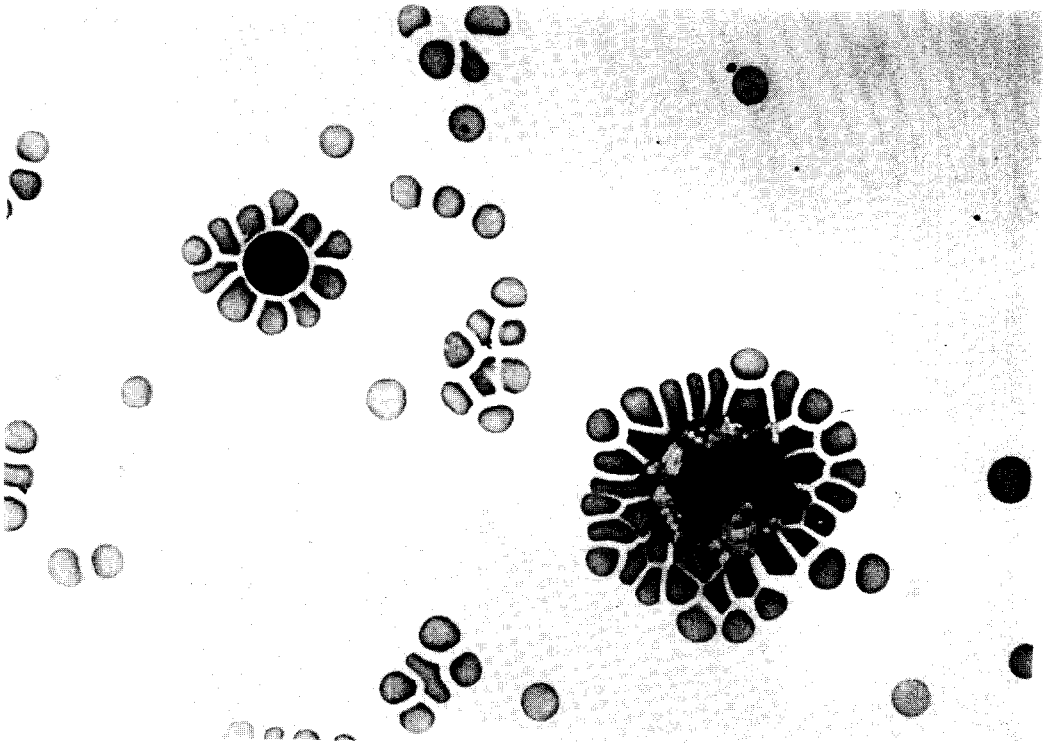

Fig. 1. *Demonstration of membrane CD4 expression by indirect rosetting. Cytocentrifuge monolayer stained with Romanowsky shows a CD4$^+$ lymphocyte and a macrophage in a serous effusion from a patient with non-haemopoietic malignancy.*