Letters

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Leu-19 in Myeloma—A New Role for an Old Antibody

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IN 1984 Uchida *et al.* [1] described strong natural killer (NK) cell activity in the bone marrow of myeloma patients when compared to normal bone marrow. These authors also reported that this increased activity was completely abrogated by an antibody with NK cell specificity (OKM 1 plus C'). Following these observations we were interested to know if a relationship exists between the number of NK cells and the extent of bone marrow infiltration by tumour cells in such cases. To our surprise, in four consecutive cases with myeloma admitted to our institution recently, high levels of strongly positive cells were found with the antibody Leu-19 (Becton-Dickinson). In order to classify these cells we performed double fluorescence experiments using the strong CD38 expression as a reference marker for plasma cells and a panel of other monoclonal antibodies with plasma cell reactivity. The results are shown in Table 1. It can be seen that Leu-19 is highly reactive with bone marrow cells from all four patients. Double fluorescence for strong CD38 expression and Leu-19 expression showed coexpression by the same cells. In one of our cases (No. 2) we tried another commercial antibody with NK cell specificity, the NKH-1 antibody (Coulter), using a direct fluorescence technique and found 42% positive cells. We then used the Leu-19 antibody for the sorting of bone marrow cells from a myeloma patient with 10% plasma cells in the mononuclear cell fraction after density gradient centrifugation. After sorting according to strong Leu-19 expression we found 80% cells with plasma cell morphology. This means an eight-fold enrichment compared to the 10% before sorting.

So, contrary to the expected result, namely to find the Leu-19 positive cells in a certain lympho-monocytoid subpopulation of the patient's bone marrow cells, we found practically all strongly expressed reactivity associated with the myeloma cells.

Using the monoclonal antibody PCA-1 which is included in the panel of antibodies shown in Table 1, alone and in combination with another antibody, Shimazaki *et al.* [2] demonstrated an effective removal of plasma cells from bone marrow cells. In autologous bone marrow transplantation for myeloma patients effective purging procedures are needed and we suggest that the Leu-19 antibody should be tested also for this purpose. The CD38 antibody, which is generally the most effective marker for plasma cells (Table 1), is also reactive with a wide spectrum of progenitor cells and therefore is not recommended for bone marrow purging.

Table 1. Flow cytometric analysis of bone marrow cells from four myeloma patients using a panel of antibodies with plasma cell specificity and the Leu-19 antibody

Patient No.	CD38*	R1-3†	IOB2	PCA-1	J5	Leu-19
1	25	23	11	4	3	16
2	48	34	27	3	1	35
3	46	15	29	21	0	34
4	37	20	19	14	8	24

*At high fluorescence intensity only.
†Kindly provided by Dr J. Katzmann, Rochester.
Numbers are percentage positive cells from two-colour analysis. Green fluorescence (R 1-3, IOB2, PCA-1, Leu-19) was gated on red fluorescence (CD38).

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2. Shimazaki C, Wisniewski D, Scheinberg D *et al.* Elimination of myeloma cells from bone marrow by using monoclonal antibodies and magnetic immunobeads. *Blood* 1988, **72**, 1248–1254.

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Differential Heat Sensitivity of Tumour Microvasculature

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RECENTLY, publications from two laboratories came to our attention that address the issue of tumour-specific differences in the sensitivity of the tumour vascular system to heat. Hill *et al.* [1] compared the thermal response, expressed as the Thermal Enhancement Ratio (TER) vs. a number of physiological parameters for eight different tumours, and concluded that a higher heat dose is required for vascular shutdown in slow growing tumours, as compared with fast growing tumours. This was

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Table 1. Spearman rank correlation coefficients for correlations between mean stoppage time and some histological characteristics

	1 ST_{50}	2 Capillary score	3 Endothelial score	4 Collagen score	5 Fibroblast score
1. ST_{50}* (min)	1				
2. Capillary score	−0.10	1			
3. Endothelial score	−0.05	0.82	1		
4. Collagen score	0.21	−0.46	−0.71	1	
5. Fibroblast score	**0.72**†	0.05	−0.24	0.68	1

In body of table: Spearman rank correlation coefficients; $n = 5$, for $2P \leq 0.05$ r should be ≥ 0.98.

*ST_{50} is mean stoppage time (in minutes) with exposure at 42.5°C [4].

†For $r = 0.72$, $2P = 0.15$.

believed to correlate with the endothelial proliferation rate which is higher in fast-growing tumours. In addition, it was found that thermoradiotherapy resulted in significant growth delay only when a reduction in perfusion of at least 60% was achieved. Hill *et al.* inferred that the fast-growing tumours would contain a more fragile and thermosensitive capillary network.

Nishimura *et al.* [2] investigated the vascular patency of four different experimental tumours by means of *post mortem* X-ray microangiography and compared this with the histologic picture. These authors concluded that differences in thermosensitivity of the various tumours was correlated with the histological structure of the tumour vasculature. More specifically, it was believed that tumour vasculature that contained perivascular connective tissue, preferably with a high number of endothelial cells, was less heat sensitive than tumour tissue having only sparse endothelial cells. No statistical evaluation was given.

Both these publications appeared after a recent report of the differential heat sensitivity of the vascular system of five different tumours [3]. In particular, the report by Nishimura *et al.* on the issue of differences in the stroma content made us look retrospectively into the histological characteristics of our tumours. A search was done on available histological slides. The yield was sufficient, but not abundant, the reason being that once a tumour has been characterized, very little new information can be added by repeated histology. The custom therefore is to follow only the tendency of a tumour to become less differentiated over the years by incidental sampling.

An arbitrary scoring system was designed, indicating the following items:

(a) a score (+, ++, +++ for respectively low, medium, high) for the impression on the density of capillaries, relative to the areas of tumour cells;
(b) a score (+, ++, +++ for respectively little, moderate, much) for an impression of the endothelial lining of the tumour capillaries;
(c) a score (+, ++, +++ for respectively little, moderate, much) for the collagen content of the tumour tissue; and
(d) a score (+, ++, +++ for respectively the presence of few, moderate or many) fibroblasts in the tumour stroma.

For diagrammatic representation and statistical analysis, the scores +, ++ and +++ were later converted into the values 1, 2 and 3. The number of slides that were available ranged from one to eight per tumour. A total of 25 slides was scored 'blindly' on two different occasions in chronological order, providing some kind of random selection.

The results of this scoring are demonstrated in Fig. 1. This picture shows that the stromal content of the five tumours, scored according to the criteria described above, differ considerably. Nonetheless, a certain trend can be discerned, i.e. that the two tumours with the highest mean stoppage time (ST_{50}) values have the highest stroma scores. However, no definite relationship can be derived from this diagram, although the general impression is that the two most heat resistant tumours with regard to the vascular system have also the highest stroma score. A Spearman rank correlation table (Table 1) shows the relevant interrelationships. It is apparent that the 'endothelial' and 'vascular' contributions have a slight negative correlation with the heat resistance. On the other hand, the score on the presence of fibroblasts shows a rather high correlation of 0.72 ($2P = 0.15$). It is therefore likely that the vascular resistance to heat depends in part on the presence of fibroblasts in the tumour tissue.

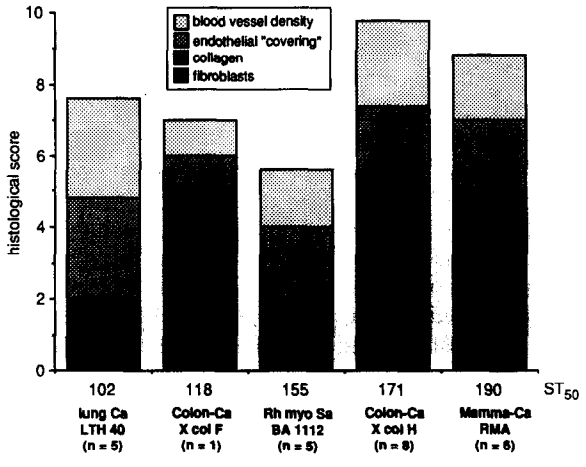

Fig. 1. Bar diagram indicating the composition of the five different tumours with respect to the four criteria. The positions on the ordinate are equally divided, but are still roughly on scale. ST_{50}; mean stoppage time at 42.5°C [4]. The tumours are: LTH40, xenografted small cell lung Ca. in immune-suppressed rats. XColF, xenografted colon Ca. in immune-suppressed rats. RhMyoSa, isogenic rhabdomyosarcoma in rats. XColH, xenografted colon Ca. in immune-suppressed rats. RMA, isogenic Mammary Ca. in rats. *n* indicates in this diagram the number of histological sections examined per tumour type. Ordinate, mean histological score.

From the statistical point of view, it should be understood that what we have been comparing here is in essence two single characteristics of five different tumours. These characteristics are both well defined. It deals on the one hand with a physiological parameter representing the heat sensitivity of the tumour vascular system and on the other hand a parameter indicating some aspects of the tumour histology. The value representing the heat sensitivity of the tumour vascular system has, for the various tumours, been determined on the basis of an extensive series of determinations performed over the last 10 years [3]. The other is a histologic characterization of the tumour vascular stroma. Therefore, in comparing these two characteristics on five tumours, the number of items entered in the Spearman rank correlation table is by definition limited to $n = 5$, and this number cannot be increased by analysing more histological slides. The same holds for the physiological parameter, i.e. the ST_{50} value. This limits the statistical power one can derive from such an analysis, and in this situation only correlation coefficients exceeding 0.98 can (with $n = 5$) be regarded to be statistically significant.

Therefore, only a trend can be derived from these data, that it is likely that the presence of fibroblasts in the tumour tissue exerts some protective effect on heat induced collapse of the tumour vasculature. The results agree to a certain extent with the observations by Nishimura *et al.* [2] and Hill *et al.* [1], that differences in heat sensitivity of the tumour vascular system are also reflected in histology and in the response to thermoradiotherapy.

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Carcinoma of the Small Intestine Following Treated Acute Myeloid Leukaemia

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LONG-TERM survivors of childhood malignancies are at greater risk of developing a second tumour probably resulting from the treatment of the primary disease. We report the case of a 19-year-old male who developed an adenocarcinoma of the small intestine 13 years after completing chemotherapy for acute myeloid leukaemia.

Our patient presented to Yorkhill Hospital in 1972 at 3 years of age with acute myeloid leukaemia (AML). Induction therapy consisted of rubidomycin, cytosine and thioguanine followed by 3 years maintenance therapy with 6-mercaptopurine, thioguanine and cytosine. The spleen was enlarged at diagnosis, became impalpable with treatment but again became palpable at 2 cm below the costal margin in 1976. Iron deficiency anaemia (Hb 11.1 g/dl, MCV 76 fl) developed in 1983. Barium swallow, meal, enema, sigmoidoscopy, colonoscopy and technetium scan were negative but oesophagoscopy showed small varices at the cardia. On oral iron, haemoglobin rose to 14 g/dl. He was transferred to the Western Infirmary in 1985 and at that time anaemia recurred with poor response to oral iron attributed to lack of compliance.

In 1988, increasing splenomegaly (5 cm) and fall in haemoglobin to 9.8 g/dl prompted further investigation. Ultrasound showed a normal liver and a patent portal vein. Liver biopsy was normal and endoscopy showed moderate sized oesophageal varices.

A diagnosis of non-cirrhotic portal hypertension was made possibly due to previous treatment. The varices were treated by injection sclerotherapy and oral iron was recommenced; however, haemoglobin only rose to 11 g/dl. In August 1988, he developed a painful, tender inflamed swelling in his right groin. Ultrasound scan was normal. The swelling settled with intravenous flucloxacillin but recurred several weeks later. On admission he was found to have a fistula with a faeculent discharge. Barium enema demonstrated deformity of the terminal ileum with a fistula running from the terminal ileum to the skin surface. At laparotomy, tumour of the terminal ileum extending along the fistulous tract was found. A right hemicolectomy was performed with a sutured ileotransverse anastomosis. The fistula was excised and the resulting skin defect sutured. Histology showed a moderately differentiated mucoid adenocarcinoma arising from a severely dysplastic tubulovillous adenoma and four lymph nodes were replaced by mucin. Despite systemic chemotherapy and radiotherapy to the fistula tract, he developed recurrent tumour at the fistula site and an ulcerating mass in his right loin. He died from widespread abdominal tumour 11 months after the diagnosis.

Small bowel malignancies are rare, accounting for 0.2% of all malignancies in the U.S.A. [1] and 1–2% of all gastrointestinal malignancies [2]. Only eight cases of adenocarcinoma of the small bowel have been reported in patients under 20 years of age. Survivors of childhood cancer experience a greater risk of developing a further tumour than expected from the rates of malignant disease in the general population. Estimates of the cumulative risk of developing a new cancer vary—3.3% at 20 years [3], 4% at 20 years [4], 12% during the period 5–20 years after diagnosis [5]. Although genetic factors may be involved in some cases, treatment for the original tumour is likely to be the most important mechanism. Patterns of second neoplasms appear to be changing with an increase in the number of children

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