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Acute leukaemia in children : aspects of diagnosis and treatment

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Possible link between unique chemokine and homing receptor expression at diagnosis and relapse location in a patient with childhood T-ALL

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Abstract

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Childhood acute lymphoblastic leukaemia (ALL) is often associated with extramedullary infiltration by leukaemic cells at diagnosis or at relapse. To understand the mechanisms behind the dissemination of T-ALL cells this study investigated the homing receptor expression on the blast cells of 11 paediatric T-ALL patients at diagnosis. One patient revealed a unique profile with high expression of the chemokine receptor CCR9 and the integrin CD103 on the T-ALL cells. Both these molecules are specifically associated with homing to the gut. This finding was clinically significant as the patient later suffered a relapse which was confined to the gut. Immunohistochemistry revealed that the leukaemic cells in the gut still expressed CCR9 and co-localized with a high expression of the CCR9 ligand, CCL25. These findings suggest that the original expression of CCR9 and CD103 on the leukaemic cells contributed to the relapse location in the gut of this patient.

Introduction

ALL is the most common childhood malignancy with the potential to infiltrate extramedullary sites(1,2). The homing of such malignant cells to specific sites appears to be a directed rather than a random event(3-6). Tumour cell migration and spread are critically regulated by chemokines and their receptors(7-10). By the preferential chemokine expression in particular sites, they direct the specific homing of haematopoietic cells. Thus the expression of chemokine receptors by malignant cells provides a marker which may predict the tropism of these cells for particular destinations.

We report the case of a 4-year-old child who at diagnosis presented with a T-ALL which subsequently switched to a clonally-related acute myeloid leukaemia (AML) during treatment. The relapse of leukaemia was confined to the gut. Interestingly the leukaemic cells of this patient displayed at diagnosis a high level of the gut homing molecules CCR9 and CD103. This rare finding is highly suggestive of a role for this receptor in determining the location where the relapse occurred.

Patients and Methods

Patients

Peripheral blood and bone marrow samples of 11 children with T-ALL, including the index case 7125, and one acute myeloid leukaemia (AML, M5) patient were obtained from the Dutch Childhood Oncology Group, The Hague, NL. Leukaemia diagnosis was based on evaluation according to the French-American-British (FAB) criteria.

Flow cytometry

Chemokine receptor antibodies (CCR1-9, CXCR1-6) were all from R&D Systems (Abingdon, UK) except CCR4, CCR7 and CXCR3 which were from BD Pharmingen (San Diego, CA, USA) and CCR8 from Alexis (San Diego, CA). The CLA and CD103 antibodies were from

BD Pharmingen and Immunotech (Marseille, France) respectively. Unlabeled antibodies were visualised using appropriate FITC-conjugated goat anti-mouse or rabbit anti-goat isotypes. For triple staining, PerCP-Cy5.5-labeled CD3 (BD PharMingen) and/or PE-labeled CD7 (Coulter, Westbrook, ME) were used as appropriate. Isotype-matched antibody controls (Southern Biotechnology Associates Inc., Birmingham, AL) were used to define detection thresholds. Events were collected using a FACSCalibur (BD Biosciences, Mountain View, CA) and analysed using CELL QUEST software.

Calcium mobilisation

Chemokine receptor activation was assessed by real-time measurement of intracellular Ca²⁺ changes using Fluo-3 according to the manufacturer's instructions (Molecular Probes, Leiden, NL) and monitored using a Perkin Elmer spectrometer LS 50B. Chemokines were used as indicated in Fig.2. Ionomycin was used as a positive control.

Immunohistochemistry

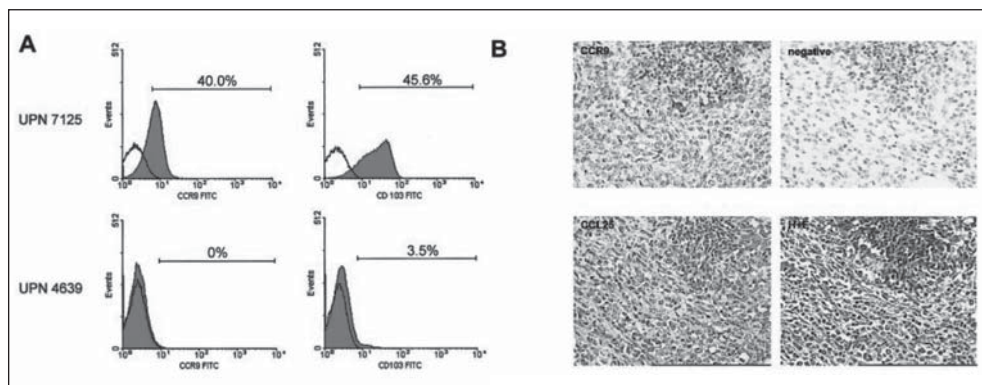
Paraffin sections of 4 µm taken from the ileum of the CCR9-positive T-ALL patient at relapse (UPN 7125) were pre-treated as described before(11) and stained using goat anti-human CCR9 antibody (Capralogics, Hardwick, MA), mouse anti-human CCL25/TECK antibody (R&D Systems) or an isotype-matched antibody (DAKO, Cambridge, UK). Immunostaining was performed using either StreptABComplex/HRP (DAKO) and DAB or EnVision/HRP (DAKO) and VECTOR NovaRed (VECTOR Laboratories, Burlingame, CA).

Results and Discussion

Flow cytometric analysis using a panel of chemokine receptors and other homing molecules (e.g. CLA and CD103) was performed on peripheral blood blast cells from 11 T-ALL patients at diagnosis. For 10 of these patients, expression of chemokine receptors on the blast cells did not differ significantly from that seen on T cells of healthy age-matched controls (data not shown). However, the T-ALL cells from one patient (UPN 7125) displayed a unique

expression profile. The gut homing molecules, CCR9 and CD103 ($\alpha\beta 7$), were expressed highly by at least 40% of the T-ALL cells (Fig. 1A). The expression of these two receptors was extremely low on the T-cells from the other 10 T-ALL patients (Fig. 1A) and healthy controls (data not shown). This unique homing receptor profile on the leukaemic cells at diagnosis correlated with the rare clinical presentation of a gut relapse in patient 7125, 18 months later. None of the other patients studied suffered a similar relapse. It seems that the level of CCR9 expression on these childhood T-ALL may be lower than that found in a recent publication(12). This could be explained by the fact that Qiuping et al. focus on CD4+ T cells and of course the age of the patients may also affect these results.

The clinical course of patient 7125 was further uniquely defined by a lineage switch (confirmed by sequencing of clonal TCR gene rearrangements) to an AML at relapse. Immunohistochemistry showed that the AML cells in the gut still expressed the CCR9 receptor (Fig. 1B). High expression of the corresponding ligand, CCL25/TECK, was also detected



For full colour see figure section

Figure 1. Unique expression of CCR9 and CD103 on the leukaemic cells of patient 7125. Multi-colour flow cytometry was carried out on a peripheral blood sample obtained at diagnosis using a panel of chemokine receptor and homing molecule specific antibodies in combination with markers for T cells. A) shows the unique expression of CCR9 and CD103 on the blast cells from patient 7125 (top panel). The bottom panel shows a representative result for expression of these same receptors as found on T cells of 10 other T-ALL patients. B) Immunohistochemistry was performed on the tumour cell infiltrate in the ileum of patient 7125. Using an anti-CCR9 polyclonal antibody and DAB detection (brown) there was clear positivity of the tumour cells for CCR9. The specificity of the CCR9 staining was confirmed by omitting the CCR9 antibody as a negative control. Furthermore, immunohistochemical staining using an anti-CCL25/TECK monoclonal antibody and NovaRed detection revealed a high expression of this CCR9 ligand in the tumour mass. The lower right picture shows hematoxylin and eosin staining of the same area of the affected ileum at relapse.

(Fig. 1B). This co-localization suggests that the leukaemic cells may have contributed to the increased local expression of CCL25. The localized presence of CCL25 at relapse may not just have functioned as a retention factor for the CCR9+ leukaemic cells but may also have contributed to leukaemic cell growth and survival(13-15).

The T-ALL blasts from patient 7125 obtained at diagnosis were tested for their response to CCL25/TECK, by monitoring changes in intracellular Ca²⁺. The addition of 200 and 100 nM CCL25/TECK to the blast cells of patient 7125 resulted in a significant dose-dependent response (Fig. 2). CXCL12/SDF-1 α whose receptor, CXCR4, was also highly expressed by the T-ALL blasts induced a significant calcium flux as well. Figure 2 further shows representative results from one of three CCR9- T-ALL patients. These CCR9-, CXCR4+ T-ALL blasts consistently failed to respond to CCL25/TECK, but did respond to CXCL12/SDF-1 α .

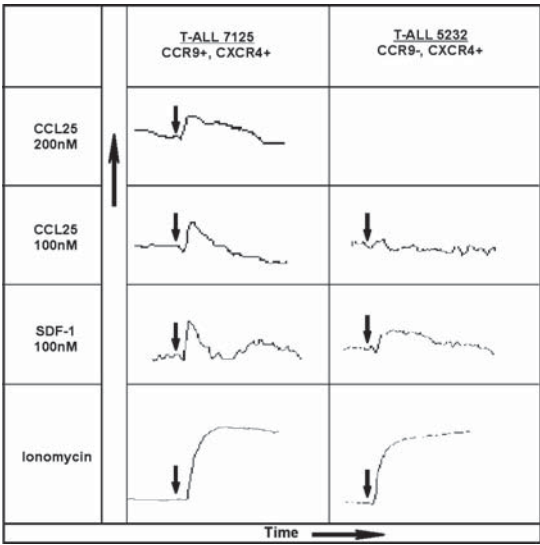


Figure 2. CCL25/TECK-induced calcium mobilization on CCR9+ T-ALL blasts

The human chemokines, CCL25/TECK (CCR9 ligand) and CXCL12/SDF-1 α (CXCR4 ligand) were tested for calcium mobilization on 2ml aliquots of 107/ml Fluo-3 AM-loaded T-ALL blasts. The left panel shows the dose-response of patient 7125 T-ALL blasts to hCCL25/TECK and the response to hCXCL12/SDF-1 α , the right panel shows the response of patient 5232 to these chemokines. The chemokine receptor expression is indicated at the top of each panel. Ionomycin was also used as a stimulator of calcium influx to obtain the maximal response. The arrows indicate the time of additions.

This study reports the finding of a unique homing receptor profile on the T-ALL cells of a paediatric patient suggesting they home to the gut. This result seems particularly relevant as this patient suffered a gut relapse 18 months after diagnosis. These findings are highly suggestive that the expression of CCR9 and CD103 on the original leukaemic cells contributed to the relapse location. Based on preliminary results of an ongoing, separate study into AML patients, CCR9 is frequently expressed on AML blasts (M4 and M5) but CD103 expression is rare (unpublished data). However, in the case of one AML patient, we did find increased expression of both molecules on the AML blasts at diagnosis. This AML patient was the only one to suffer a gut relapse, supporting our hypothesis regarding the possible role of these receptors in relapse location. The data presented provides further evidence for the link between expression of homing molecules and extramedullary organ infiltration by leukaemic cells. If performed at diagnosis, this type of analysis may identify a subset of patients with a high chance of extramedullary relapse.

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