Expression of killer cell inhibitory receptors is restricted to true NK cell lymphomas and a subset of intestinal enteropathy-type T cell lymphomas with a cytotoxic phenotype


Abstract
Background/Aims—Killer inhibitory receptors (KIR) have a modulating effect on the cytotoxic functions of natural killer (NK) cells and T cells. Because lymphoma cells often have the same receptors as their non-neoplastic counterparts, this study investigated the expression of KIR on well defined groups of NK and T cell lymphomas, with and without a cytotoxic phenotype, from different sites of origin. Methods—Nine CD56+/CD3− NK cell lymphomas, 29 CD3+/CD56− T cell lymphomas with a cytotoxic phenotype, and 19 T cell lymphomas without a cytotoxic phenotype were stained for KIR using monoclonal antibodies specific for CD94, CD158a, and CD158b. In addition, the expression of KIR was studied on normal lymphoid tissues. Results—KIR expression was seen in five of nine true NK cell lymphomas including three of four nasal, one of four cutaneous, and one of one intestinal lymphoma nasal type. Double staining for CD56 and CD94 in normal lymphoid tissues revealed that KIR was predominantly expressed by CD56+ NK cells and sporadically on CD8+ T cells. Moreover, enteropathy-type T cell lymphomas with a cytotoxic phenotype showed KIR expression (three cases expressing CD94 and one case expressing CD158a). All nodal and extranodal non-intestinal T cell lymphomas with or without a cytotoxic phenotype lacked expression of KIR. Conclusions—These results show that KIR expression is restricted to CD56+/CD3− true NK cell lymphomas originating from the nose, gut, and skin, as well as in a subset of extranodal T cell lymphomas originating from the small intestine, which possessed a cytotoxic phenotype. Thus, the presence of KIR on NK/T cell lymphomas seems to mimic the distribution of KIR found on NK and T cells in normal lymphoid tissue.

Keywords: lymphoma; granzyme B; killer inhibitory receptors; CD94

The classification of extranodal T cell and natural killer (NK) cell lymphomas is based on a combination of clinical, immunophenotypic, cytogentic, and molecular genetic features. The development of monoclonal antibodies directed against components of cytotoxic granules, such as perforin, granzyme, and T cell intracytoplasmic antigen 1 (TIA-1), has further detailed the immunophenotypic characteristics of T cell and NK cell lymphomas and led to the recognition of T cell and NK cell neoplasms with a cytotoxic phenotype. The expression of cytotoxic proteins has been demonstrated in nodal anaplastic large cell lymphomas (ALCLs), angiocentric (nasal and nasal-type) NK/T lymphomas, hepatosplenic γδ T cell lymphomas, subcutaneous panniculitis-like T cell lymphomas, intestinal lymphoma nasal-type lymphomas, NK/T cell lymphomas in immunocompromised patients, mycosis fungoides (MF), and CD30 positive lymphoproliferative disorders arising in the skin.

A new class of molecules has been discovered recently that can modulate the cytotoxic activity of NK and T cells. These killer cell inhibitory receptors (KIRs) were initially found on a subset of NK cells but subsequent studies revealed that in addition to NK cells, KIRs can also be detected on a subset of CD8 and, rarely, on CD4 positive lymphocytes. At present, two groups of KIR are known: the immunoglobulin (Ig) superfamily-like receptors (CD158a, CD158b, and NKB1), which specifically recognize human major histocompatibility complex (MHC) antigen C (HLA-C) and HLA-B alleles, and the lectin-like receptors (CD94/NKG2A), which specifically recognize self MHC peptides presented by HLA-E. In vitro studies revealed that ligation of KIR by HLA class I molecules on target cells results in inhibition of the NK cell mediated cytotoxicity or T cell receptor (TCR) mediated killing. This inhibitory effect of KIRs on cellular cytotoxicity is mediated by so called ITIMs (immunoreceptor tyrosine based inhibition motifs), which upon phosphorylation can abrogate intracytoplasmic signal transduction. Current evidence suggests that KIRs are involved in peripheral regulatory mechanisms avoiding overwhelming immune responses during strong and/or long lasting immune activation.

Earlier studies on the expression of KIRs are largely restricted to in vitro experiments and have focused on normal lymphoid subpopulations. The aims of our present study were to characterise the expression of KIRs on NK cell and T cell lymphomas. To this end, KIR...
expression was investigated on a panel of
CD56+/CD3+ NK cell and CD56−/CD3+ T cell
lymphomas, and results were correlated with
immunophenotype and the expression of cyto-
xic proteins. As a control, KIR expression on
normal lymphoid tissue was investigated.

Materials and methods

Tissues

Frozen samples of NK and T cell lymphomas
were selected from the archive of the depart-
ment of pathology of the Leiden University
Medical Centre, the department of dermatology,
University Hospital Munich. These lympho-
mas were classified according to the World
Health Organisation (WHO) classification, whereas cutaneous lymphomas were classified
according to the European Organisation for
Research and Treatment of Cancer (EORTC)
classification based on a combination of
clinical, histological, and immunophenotypical
data, as described previously.

Three groups of NK/T cell lymphomas were
investigated. The first group comprised true
NK cell lymphomas originating from the naso-
pharyngeal tract (n = 4), the skin (n = 4), and
gut (n = 1) as defined by the expression of
CD56 and absence of CD3 (table 1). In all NK
cell lymphomas the tumour cells expressed a
cytotoxic phenotype as determined by
CD8. All NK cell lymphomas without a cytotoxic
phenotype, and 12 cases of MF without a
cytotoxic phenotype. All 19 cases had a CD3+/CD8− immunophenotype

Monoclonal antibodies

Primary antibodies used for phenotyping the
lymphoma cases were CD30 (BerH2; Dako,
Glostrup, Denmark), CD3 (leu4; B&D, San
Jose, California, USA), CD4 (leu3a; B&D),
CD8 (RIV4; RIVM, De Bilt, the Netherlands),
CD56 (B&D; IgG1 subtype), CD57 (B&D),
CD68 (Dako), LMP1 (Organon Teknika,
Box-
tel, the Netherlands), granzyme B (GrB7;
Kummer and colleagues‘), and TIA-1 (Coulter
Immunology, Hialeah, Florida, USA). For the
detection of KIR, the following monoclonal
antibodies were used: CD94 (Coulter; IgG2a
subtype), CD158a (Coulter), and CD158b
(Coulter)

Immunohistochemistry

Frozen tissue sections were fixed with parafor-
maldehyde (4%, 10 minutes) and subsequently
stained using a standard three step streptavidin–biotin complex based method
with diaminobenzidine (DAB) as chromogen,
and 2 (EBER1/2) probe and immunohisto-
chemical detection of latent membrane protein
1 (LMP1).

The second group of lymphomas tested were
T cell lymphomas with a cytotoxic phenotype
defined by a CD3+ immunophenotype in com-
bination with granzyme B and TIA-1 expres-
sion. This group consisted of enteropathy-type
T cell lymphoma (n = 5), nodal CD30− ALCL
(n = 5), primary cutaneous CD30+ large T cell
lymphoma (LTCL; n = 5), lymphomatoid pa-
pulosis (LyP; n = 8), and MF (n = 6). All cases
had an CD3+/CD4− phenotype with the excep-
tion of two enteropathy-type T cell lymphoma
(CD3−CD4+) and one case of MF, which
expressed CD3 and CD8.

The third group were cutaneous T cell lymph-
as without a cytotoxic phenotype, and
comprised two cutaneous CD30+ LTCLs, five
nodal T cell lymphomas not otherwise speci-
ﬁed (NOS), and 12 cases of MF without a
cytotoxic phenotype. All 19 cases had a CD3+/CD4−/CD8− immunophenotype

Table 1  Expression of cytotoxic proteins and EBV status of neoplastic cells in nodal and extranodal lymphomas

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N</th>
<th>GrB/TIA-1</th>
<th>EBV</th>
<th>CD3</th>
<th>CD4</th>
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</tr>
</tbody>
</table>

*Only two cases tested.

ALCL, anaplastic large cell lymphoma; EBV, Epstein-Barr virus; GrB, granzyme B; LTCL, large T cell lymphoma; NK, natural killer; NOS, not otherwise specified.
incubation with horseradish peroxidase labelled goat antimouse IgG1 and biotin labelled goat antimouse IgG2a (30 minutes) for the detection of CD56 and CD94, respectively. The horseradish peroxidase was visualised by fluorescein conjugated tyramine, whereas the biotin label was detected with cy3 conjugated streptavidin.

CONTROLS
Frozen sections of lymphoid tissue (tonsils, lymph nodes, spleen, and thymus) were used as controls for the immunohistochemical detection of KIR expression. In addition, double staining for CD94 in combination with either CD56 or CD8 was performed to identify the cellular origin of the KIR expressing cells.

INTERPRETATION OF IMMUNOHISTOCHEMISTRY
The identification of neoplastic T cells was based on a combination of morphology and phenotype. In all cases, serial sections stained with monoclonal antibodies against CD3, CD4, CD8, CD56, and CD68 were used as an additional tool to differentiate the neoplastic T cells from reactive CD8+/CD56+ T/NK cells and dendritic cells/macrophages. Cases were scored positive when > 50% of morphologically recognisable neoplastic cells stained positive.

Results
CONTROL TISSUE
In all lymphoid tissue tested (tonsil, lymph nodes, thymus, and spleen) KIR positive cells were identified. KIR positivity (either CD94, CD158a, or CD158b) was seen as membranous staining in T cell areas (fig 1). Positive cells comprised up to 5% of the total number of lymphocytes. In all cases, there were more CD94 positive cells than CD158b positive cells, which in turn outnumbered the CD158a cells. Double staining for CD56 and CD94 demonstrated co-expression of these molecules in most CD94 positive cells, but colocalisation of CD8 with CD94 was limited to a few scattered cells (fig 2), indicating that KIRs are predominantly found on NK cells and sporadically on CD8+ T cells.

TRUE NK CELL LYMPHOMAS
KIR expression was found in five of nine true NK cell lymphomas (table 2). The expression of CD94 was detected in five of nine cases, whereas the expression of CD158a was limited to three of nine cases and the expression of CD158b was found in only one of nine cases.

In all cases positive for CD94, CD158a, or CD158b a strong membranous staining was seen in nearly all the tumour cells. The expression of multiple KIRs was seen in three cases, two nasal NK lymphomas (fig 3) and one case of intestinal lymphoma, nasal type. In two cases, a combination of CD94 and CD158a was detected and in one case a combination of CD94 with both CD158a and CD158b was found. The proportion of cases expressing KIRs varied between different localisations of NK lymphomas. KIR expression was detected in the one case of intestinal lymphoma, nasal type, in three of four nasal NK/T cell lymphomas, and in one of four cutaneous NK cell lymphomas.

Scattered CD94, CD158a, or CD158b positive lymphocytes in the reactive infiltrate were seen in all cases.
Table 2  Expression of killer cell inhibitory receptor in nodal and extranodal lymphomas

<table>
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<tr>
<th>Diagnosis</th>
<th>N</th>
<th>CD94</th>
<th>CD158a</th>
<th>CD158b</th>
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<td>Intestinal lymphoma, nasal type</td>
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<td>T cell lymphomas with a cytotoxic phenotype</td>
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<td>Enteropathy-type T cell lymphoma</td>
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</table>

ALCL, anaplastic large cell lymphoma; LTCL, large T cell lymphoma; NK, natural killer; NOS, not otherwise specified.

T CELL LYMPHOMAS WITH A CYTOTOXIC PHENOTYPE

Despite the presence of a cytotoxic phenotype in the enteropathy-type T cell lymphomas (n = 5), nodal CD30+ ALCLs (n = 5), primary cutaneous CD30+ LTCLs (n = 5), LyPs (n = 8), and MFs (n = 6), CD94 expression was detected in only four cases of enteropathy-type T cell lymphoma, and in the other T cell lymphomas CD94, CD158a, or CD158b expression was not found. In these biopsies, the reactive lymphocytes served as a useful internal control, showing scattered CD94+ and CD158b+ cells in up to 5% of the total infiltrating lymphocytes.

T CELL LYMPHOMAS WITHOUT A CYTOTOXIC PHENOTYPE

In the two cutaneous CD30− LTCLs, five nodal T cell lymphomas NOS, and 12 cases of MF without a cytotoxic phenotype, KIR expression was not detected on the neoplastic T cells. Again, the reactive lymphocytes served as a useful internal control, showing scattered CD94+ and CD158b+ cells in up to 5% of the total infiltrating lymphocytes. However, CD158a expression was not detected on reactive cells.

Discussion

Non-Hodgkin’s lymphoma (NHL) cells are considered to be neoplastic counterparts of functional, recirculating, site restricted lymphocytes and as such have many characteristics in common with their normal counterparts. The main finding of our present study is that the expression of KIRs, a new class of MHC class I specific receptors, is restricted to true NK cell lymphomas. We demonstrate the expression of KIRs in CD56+/CD3− NK cell lymphomas arising in the nasopharyngeal tract, and in intestinal lymphomas, nasal-type lymphomas, and primary cutaneous NK cell lymphomas.

Expression of CD94 was more frequently detected (five of nine cases) than CD158a (three of nine cases) and CD158b (one of nine cases), similar to the staining pattern of KIRs in normal NK cells in lymph nodes, tonsils, spleen, and thymus. In addition, combinations of CD94 with CD158a and CD158b were detected in a few cases. Interestingly, in a study by Mingari et al an identical pattern of KIR expression was described in blood derived NK cells as determined by fluorescence activated...
cell sorter (FACS) analysis. Taken together, these data further support the concept that the neoplastic cells of lymphomas with a CD56+/CD3− NK cell phenotype in the nasopharyngeal tract, the gastrointestinal tract, and skin all originate from NK cells.

In contrast to true NK cell lymphomas, the expression of CD94 was also found on four cases of enteropathy-type T cell lymphoma with a cytotoxic phenotype. Again, this is in line with in vitro experiments where chronic activation of T cells induces the expression of cytotoxic proteins in a subset of T cells in combination with the expression of KIRs on a few of these lymphocytes. Our data are in line with the study of Haedicke et al, in which three T cell lymphomas with a cytotoxic phenotype originating from the small intestine showed expression of CD94. One of these cases was a γδ T cell lymphoma arising from the spleen. Because of the lack of frozen material, no γδ T cell lymphomas were included in our study.

Previous studies showed that NK cells can express several members of the KIR family at different NK cell subsets, probably with distinct functions. One of these cases was a γδ T cell lymphoma arising from the spleen. Because of the lack of frozen material, no γδ T cell lymphomas were included in our study.

In conclusion, our study reveals that KIR expression is restricted to CD56+CD3− true NK cell lymphomas originating from the nose, gut, and skin, as well as in a subset of extranodal T cell lymphomas originating from the small intestine, which possessed a cytotoxic phenotype. Thus, the presence of KIRs on NK/T cell lymphomas seems to mimic the distribution of KIRs found on NK and T cells in normal lymphoid tissue.

We thank Professor Ph M Kluin and Dr A Neeffes-Borst for providing material. Professor A Chott is gratefully acknowledged for helpful discussions.