

Review

Natural killer cell malignancies: clinicopathologic and molecular features

L.L.P. Siu, J.K.C. Chan and Y.L. Kwong

Department of Medicine, Queen Mary Hospital and Department of Pathology, Queen Elizabeth Hospital, Hong Kong

Summary. Malignancies of natural killer (NK) cells have increasingly been recognized as distinct clinicopathological entities. The tumor cells are characterized by an immunophenotype of CD2+, surface CD3-, cytoplasmic CD3 +, and CD56+. The T cell receptor gene is in germline configuration, and a consistent association with Epstein-Barr virus is demonstrable. Pathologically, the tumor cells show variable cytological appearances, with frequent angioinvasion and angiocentricity associated with zonal necrosis. Clinically, most cases affect the nasal cavity or other parts of the upper aerodigestive tract, and are referred to as nasal NK cell lymphoma. A minority involve extranasal sites such as the skin, gastrointestinal tract and testis, and are often referred to as extranasal NK cell lymphoma. A particularly aggressive form presents fulminantly as disseminated disease, sometimes with a leukemic phase, and is referred to as aggressive NK cell lymphoma/leukemia. Cytogenetic and molecular analysis have shown DNA losses at chromosomes 6q, 11q, 13q and 17p to be recurrent aberrations in NK cell malignancies. Frequent DNA gains are also found in chromosomes 1p, 6p, 11q, 12q, 17q, 19p, 20q, and Xp. These regions of DNA losses and gains should be targets for further investigation in order to understand the molecular pathogenesis of this lymphoma. Finally, optimal treatment modalities need to be determined, as all subtypes of NK cell malignancies are associated with a poor prognosis.

Key words: CD56, Natural killer cell, Lymphoma, Leukemia

Introduction

Natural killer (NK) cells, first recognised during the course of studies on cell mediated cytotoxicity, were defined functionally as cytolytic cells capable of lysing

target cells without major histocompatibility complex (MHC) restriction. Since its original functional definition, much has been learned of its phenotypic, immunologic and biologic characteristics. Furthermore, malignancies of NK cells are increasingly recognized. These malignancies show interesting pathologic and biologic features, which may provide an important model for the study of NK cells in health and disease.

Natural killer cells – ontogeny and phenotype

Natural killer (NK) cells are cytolytic cells capable of killing a wide variety of target cells, including tumor cells and cells infected with bacteria and viruses. Morphologically, NK cells are lymphocytes showing abundant pale cytoplasm with azurophilic granules, which contain cytolytic molecules such as perforin, granzyme B, and TIA-1 (Spits et al., 1995; Mori et al., 1996; Ng et al., 1997). Immunophenotypically, NK cells show a variable expression of the T lineage-associated antigens, such as CD2, CD7 and CD8 (Robertson and Ritz, 1990; Oshimi, 1996). They are typically negative for surface-CD3 (most commonly assayed using the monoclonal antibodies Leu4 or OKT3), but they do express cytoplasmic CD3 chain. Fetal NK cells also express the α and β chains of the CD3 molecule in the cytoplasm. In adult NK cells, cytoplasmic CD3 can be acquired upon activation (Lanier et al., 1992; Spits et al., 1995). NK cells express the γ chain of interleukin-2 receptor and variably a number of “NK-associated markers”, including CD16 (Leu11b), CD56 (NKH1, Leu19) and CD57 (Leu7, HNK1). Among these three markers, CD56 is the most consistently expressed, and has been designated as a marker for natural killer cells. However, CD56 is not entirely specific for NK cells, and can also be expressed on NK-like T cells, neural and neuroendocrine tissues, and sometimes on skeletal muscle (Kern et al., 1993).

The bone marrow is the main site for NK cell development. The thymus is not absolutely essential for NK cell development, since thymus deficient animals have normal numbers of NK cells. Nonetheless, NK cells can develop in the thymus, since T/NK progenitors

and mature NK cells can be demonstrated in the organ. The interleukin-7 complex is probably crucial for NK cell development (Spits et al., 1995). Although NK cells represent a separate lineage of lymphocytes distinct from T cells, cells of these two lineages are developmentally closely related. There exists a bipotential T/NK progenitor, which can either commit to the NK cell lineage (without rearrangements of the TCR genes) or alternatively develop into the T cell lineage (with rearrangements of the TCR genes) (Sanchez et al., 1993, 1994). The bipotential T/NK progenitor has a phenotype of CD34⁺⁺ CD33⁺⁺ CD7⁺⁺ CD2[±] CD5[±] CD1⁻ cCD3⁺. Commitment to the NK cell lineage causes the loss of CD34, CD33 and CD5, and the acquisition of CD56.

NK cell receptors

NK cells do not express T cell receptor or Ig, but express receptors for MHC class I antigens (Lanier,

1998). The CD94/NKG2 receptors are disulphide-bonded heterodimers that belong to the C-type lectin superfamily. The CD158a and CD158b are members of killer-cell inhibitory receptors (KIR) that belong to the immunoglobulin (Ig) superfamily. Both type of receptors are expressed on normal NK cells and a subset of T cells. Receptor ligand binding mediates inhibition of NK cell function. It has been proposed that the CD94/NKG2 receptors represent a more ancestral type of MHC class I recognition strategy than the CD158a/CD158b receptors, which may have evolved later to provide a more refined system of MHC recognition (Moretta, 1997). Expression of CD94 and KIR has been demonstrated in malignancies of putative NK cell lineage (Haedicke et al., 2000; Dukers et al., 2001).

Natural killer cell malignancies

In the 1994 Revised European-American Lymphoma (REAL) classification, NK cell malignancies were still

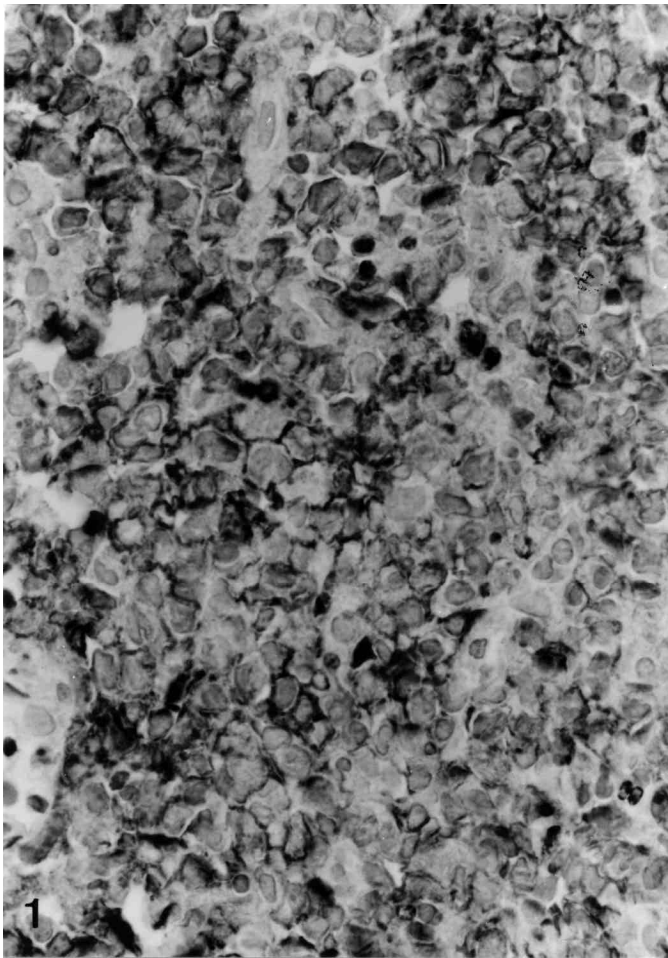


Fig. 1. Immunohistochemical staining shows cell membrane staining for CD56. x 200

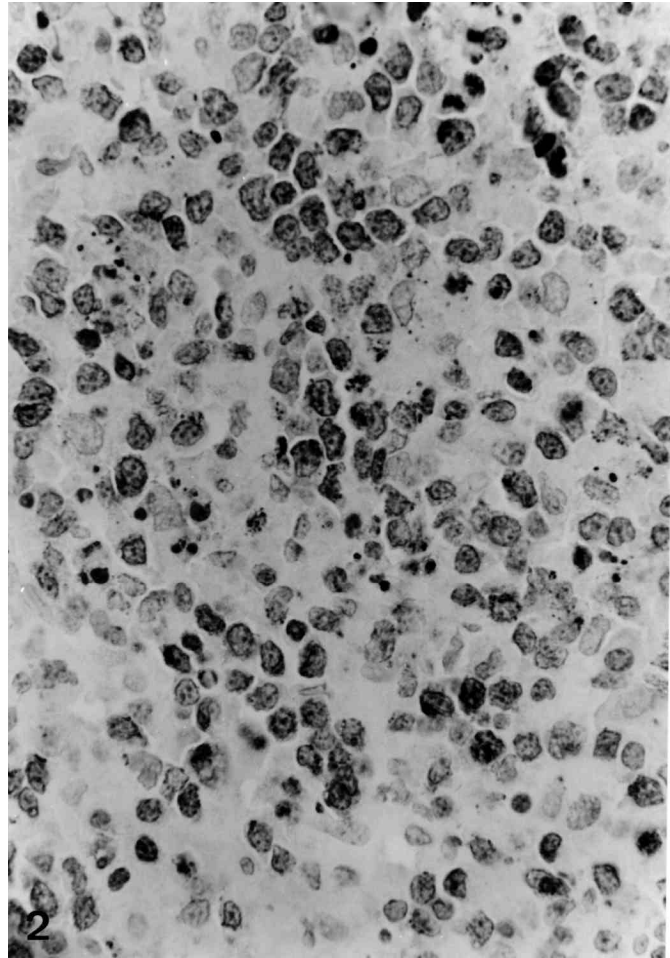


Fig. 2. Immunohistochemical staining shows granular cytoplasmic staining for granzyme B. x 200

Natural killer cell malignancies

given the non-specific designation 'angiocentric lymphoma' (Harris et al., 1994; Chan et al., 1995). Their distinct nature was formally acknowledged at a slide workshop on nasal and related extranodal angiocentric T/NK cell lymphomas (Jaffe et al., 1996). In the new World Health Organization (WHO) classification scheme, the disease is classified as NK/T cell lymphoma and aggressive NK cell leukemia (Jaffe et al., 2001).

NK cell neoplasms are characterized by an immunophenotype of CD2+, surface CD3-, cytoplasmic CD3+, CD56+, and T cell receptor (TCR)- (Figs. 1, 2). Typically, the T-cell receptor gene is in germline configuration (Suzumiya et al., 1994; Chiang et al., 1996; Emile et al., 1996). Some studies have reported surface CD3 positivity in some cases of NK cell lymphomas, but more careful immunological analysis often shows that the CD3+ cells are merely reactive cells (Tao et al., 1995), implying that the reported CD3 positivity might be due to misinterpretation of the staining of reactive T cells in the background (Suzumiya et al., 1994; Chan et al., 1996). However, the tumor cells

are almost invariably stained by the polyclonal CD3 antibody or monoclonal antibody PS1 (Aozasa et al., 1995; Chan et al., 1995; Steward et al., 1997). The discordant CD3 staining in fresh/frozen (surface negative) versus paraffin-embedded (cytoplasmic positive) tissues can be explained by the differences in the reactivities of the CD3 antibodies. The monoclonal antibodies such as Leu4, T3 and OKT3 recognize a conformational epitope formed by the various chains of the complete CD3 complex, either CD3 ϵ or CD3 δ (Salmeron et al., 1991) while the polyclonal CD3 antibody or monoclonal antibody PS1 is directed against the CD3 γ chain. Since NK cells express only CD3 γ chain or the incomplete CD3 molecule including CD3 γ in the cytoplasm but not the full CD3 complex, they are stained in paraffin sections by polyclonal CD3 antibodies or PS1, but not in fresh/frozen tissues by Leu4, T3 or OKT3. This feature provides an important distinction between NK cells and T cells on immunohistochemical studies of lymphomas. Another characteristic feature of NK cell lymphoma is the very strong association with Epstein-Barr virus (EBV) (Weiss et al., 1994; Tao et al., 1995), which can be detected in the tumor very sensitively by in situ hybridization (ISH) for EBV encoded RNA (EBER) (Fig. 3).

Clinically, NK cell malignancies comprise several distinct clinical syndromes: (1) nasal NK cell lymphoma, (2) nonnasal or nasal-type NK cell lymphoma, and (3) aggressive NK cell lymphoma/leukemia (Table 1) (Jaffe et al., 1996). These three entities show an identical immunophenotype and EBV association. Nonetheless, occasional cases may exhibit overlapping features. Rarely, NK cell malignancies may arise in immunocompromised patients after solid organ transplantation (Hsi et al., 1998; Kwong et al., 2000; Mukai et al., 2000).

Nasal NK cell lymphoma

In nasal NK cell lymphoma, the median age of presentation is in the fifth decade, with a male to female ratio of approximately 3:1. It is the commonest lymphoma type among primary lymphomas of the nasal cavity. According to one study, more than 50% of the lymphomas affecting the nasal cavity with or without simultaneous involvement of the nasopharynx are of NK/T cell lineage (Cheung et al., 1998). Others have also reported similar findings concerning the relationship of lineage with anatomic location for lymphomas of the upper aerodigestive tract (Noorduyn et al., 1991; Ye et al., 1992). In the past, the disease had been variably referred to as lethal midline granuloma, polymorphic reticulosis and other more obscure terms such as progressive lethal granulomatous ulceration, nonhealing granuloma and malignant midline reticulosis (Stewart, 1933; Eichel et al., 1966; Kassel et al., 1969). Based on immunophenotypic and genotypic results, many of the cases of lethal midline granulomas and nasal lymphomas have now been shown to be of putative NK cell origin,

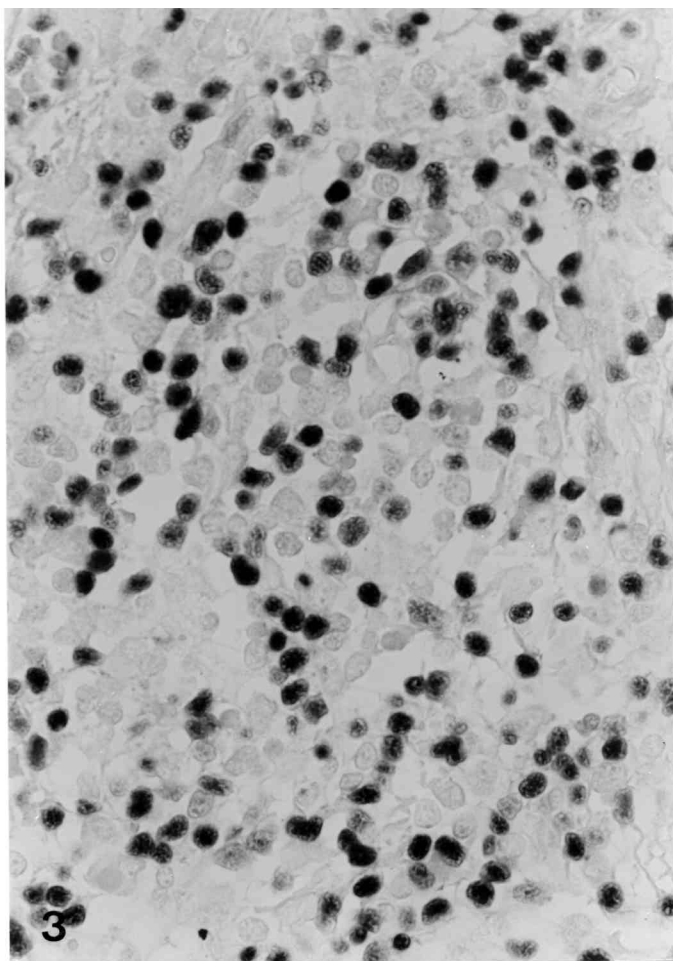


Fig. 3. In-situ hybridization for EBV shows strong nuclear labeling. x 200

with an immunohistochemical profile of CD2+, surface CD3 (Leu4)-, CD56+ (Ho et al., 1990; Ng et al., 1997) and the T-cell receptor in germline configuration (Emile et al., 1996).

Clinically, patients present with a mass lesion or relentless progressive ulceration and destruction of the midline facial structures (Fig. 4). Common symptoms include nasal discharge, nasal obstruction, purulent rhinorrhea, epistaxis and local swelling of the nasal bridge. In more advanced cases, there may be erythema, swelling of the face, proptosis and nasal septal perforation (Davison et al., 1996; Cheung et al., 1998). Hemophagocytic syndrome complicates the disease at initial presentation or during the clinical course in about

10% of patients. Lymph node involvement at presentation is uncommon (Cheung et al., 1998), and involvement of distant sites rare (Kwong et al., 1997a). Therefore, most patients have an early stage disease (stage I/II, 82%) at presentation.

Histologically, the tumor is characterized by a partially or extensively ulcerated mucosa densely infiltrated by abnormal lymphoid cells (Figs. 5-8). There are often large areas of zonal cell death. The cytologic spectrum is broad. Tumor cells may be predominantly small cells (found in about 8% of cases); mixed small and large cells (49% of cases); or predominantly large cells (43% of cases) (Cheung et al., 1998). The small cells have irregularly folded, angulated, and sometimes

Table 1. Clinicopathologic features of nasal NK cell lymphoma, extranasal NK cell lymphoma, and NK cell lymphoma/leukemia.

	NASAL NK CELL LYMPHOMA	NON-NASAL NK CELL LYMPHOMA	NK CELL LYMPHOMA/LEUKEMIA
Sex	M > F	M > F	M > F
Median age	50 – 60 years	50 – 60 years	30 – 40 years
Main site of involvement	Nasal, upper aerodigestive tract	Skin, gastrointestinal tract, testis, other soft tissues	Disseminated, liver, spleen, bone marrow, lymph nodes
Histological features	Variable cell size, nuclear folding, necrosis and angiocentricity	Variable cell size, nuclear folding, necrosis and angiocentricity	Usually monotonous malignant cells, cytoplasm may contain azurophilic granules. Angiocentricity and necrosis may be seen in organ infiltrates.
Immunophenotyping	CD2+, CD3/Leu4-, CD3 +, CD7±, CD56+, CD16-	CD2+, CD3/Leu4-, CD3 +, CD56+, CD16-	CD2+, CD3/Leu4-, CD3 +, CD56+, CD16±
TCR	Germline	Germline	Germline
EBV	Most cases, clonal	Most cases, clonal	Most cases, clonal
Outcome	Aggressive, median survival <12 months	Highly aggressive, median survival < 4 months	Extremely rapid fatal course, median survival < 2 months

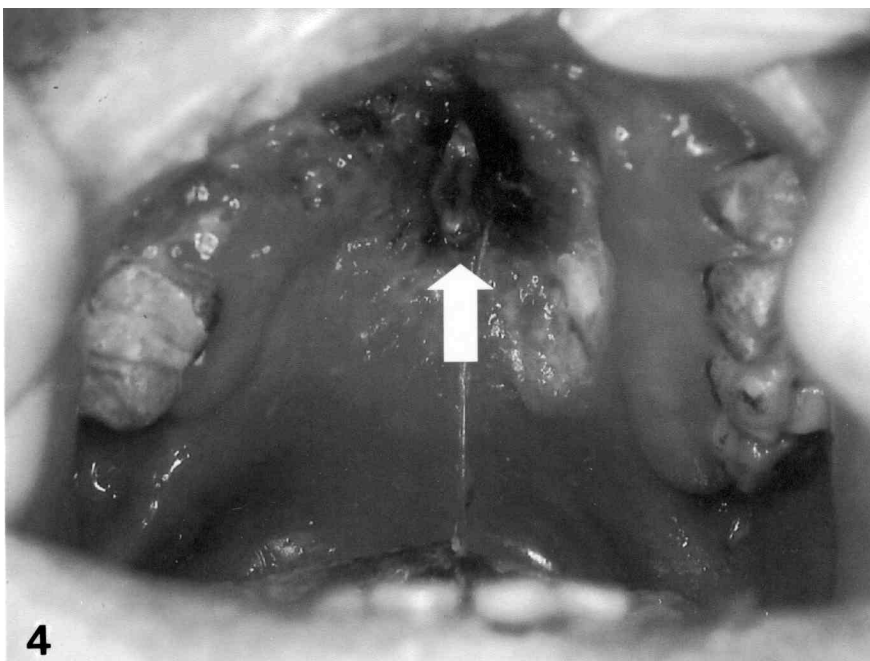


Fig. 4. A nasal NK cell lymphoma eroding through the upper palate.

serpentine-shaped nuclei with fairly dense to granular chromatin and inconspicuous nucleoli. The medium-sized cells have round or folded nuclei, granular chromatin and small nucleoli. The large cells have usually vesicular or granular chromatin and distinct nucleoli. Tumor cells possess a moderate amount of pale to clear cytoplasm, although the large cells can possess basophilic or amphophilic cytoplasm. Apoptotic bodies are frequently interspersed among the lymphoma cells. Mitotic figures are often easy to find (Figs. 9-10). In Giemsa-stained cytologic preparations, a few to many fine azurophilic granules can be seen in the cytoplasm of the tumor cells (Ho et al., 1990; Aozasa et al., 1995; Emile et al., 1996; Chan, 1998). Angiocentric and angiodestructive growth is commonly found but this phenomenon is not invariably identified, probably due to a sampling effect in the small biopsies (Fig. 11) (Suzumiya et al., 1994; Jaffe et al., 1996).



Fig. 5. Biopsy of nasal NK cell lymphoma. As typical of this neoplasm, the surface (upper field) is ulcerated and covered by fibrinous exudate mixed with necrotic tissue. The floor is densely infiltrated by abnormal lymphoid cells. The right lower field shows a blood vessel with fibrin deposition in the wall, a common phenomenon in NK cell lymphoma. x 50

Extranasal NK cell lymphoma

Extranasal NK cell lymphomas represent the counterpart of nasal NK cell lymphomas occurring in sites other than the nasal cavity and nasopharynx. These lymphomas are sometimes referred to as “non-nasal” or “nasal-type” NK/T cell lymphomas. This group of neoplasms were simultaneously characterized by Wong et al. (1992) and Kern et al. (1992) as a peculiar form of lymphoma involving extranodal and unusual sites that showed frequent angiocentricity. In Chinese patients where most of the cases have been reported, the incidence of extranasal NK/T cell lymphoma is approximately half that of nasal NK/T cell lymphoma. Similar to nasal tumors, the median age is in the fifth decade with a male to female ratio of 3:1 (Chan et al., 1997). Like nasal NK cell lymphoma, extranasal NK cell lymphomas show similar cytologic features, immunophenotype and genotypic profile. There is also a strong association with EBV (Kern et al., 1992; Nakamura et al., 1995; Chan et al., 1997; Drenou et al., 1997).

Extranasal NK cell lymphoma often involves multiple anatomic sites in the absence of superficial lymphadenopathy. There is a predilection for the skin, upper aerodigestive tract (such as palate and larynx), gastrointestinal tract, testis, spleen, soft tissues (especially muscle), central nervous system, lung, and liver (Kern et al., 1992; Wong et al., 1992; Chan et al., 1997). Most patients have stage III or IV disease at presentation. Systemic symptoms such as fever, malaise and weight loss are common. The serum lactate dehydrogenase level is commonly elevated.

Aggressive NK cell leukemia/lymphoma

Aggressive NK cell leukemia/lymphoma was first characterized by Imamura and colleagues (1988, 1990). This entity is often designated as “leukemia/lymphoma” rather than simply “leukemia” or “lymphoma”, because features of both forms may be concomitantly present. Peripheral blood involvement is common, but the percentage of circulating tumor cells can be low. The marrow is involved, but in contrast to the usual leukemias, the infiltration can be subtle and focal. In the REAL classification, the disease is categorized as large granular lymphocyte leukemia, NK-cell type. In the WHO classification, it is recognized as a distinct entity. Aggressive NK cell leukemia/lymphoma typically affects young to middle-aged adults. There is equal sex incidence or slight male predominance (Imamura et al., 1990; Chan et al., 1997). Patients are usually very ill at presentation, with fever, systemic symptoms, deranged liver function, hepatosplenomegaly, sometimes accompanied by systemic lymphadenopathy. In contrast to extranasal NK cell lymphoma, skin lesions are uncommon. Some cases may be complicated by a reactive hemophagocytic syndrome (Okuda et al., 1991; Chan et al., 1997). Rare cases manifest as an abrupt

transformation from an indolent (chronic) NK-cell large granular lymphoproliferative disorder (Ohno et al., 1988, 1989). The prominent systemic symptoms, younger age of onset, widespread tissue involvement with an invariable marrow infiltration (nasal/extranodal NK cell lymphomas rarely disseminate, even terminally), and the fulminant disease course suggest that aggressive NK cell lymphoma/leukemia may represent a distinct clinical entity, and not merely a late manifestation of localized NK cell lymphoma at an advanced stage (Soler et al., 1994). The disease pursues a highly aggressive course, resulting in death within a short time (Imamura et al., 1990; Chan et al., 1997; Kwong et al., 1997a).

Blood counts typically reveal anemia, leucopenia and thrombocytopenia, associated with a variable degree of lymphocytosis. The total white cell count may be raised, normal or low. Tumor cells resembling large granular lymphocytes account for a few percent to >80% of all leukocytes (Imamura et al., 1990; Chan et al., 1997) (Fig. 12a). The neoplastic cells possess round nuclei with condensed chromatin, or larger nuclei with

mildly irregular foldings. In some cases, nucleoli can be prominent in a proportion of cells. The cytoplasm is lightly basophilic, and contains fine or coarse azurophilic granules. Hemophagocytosis may sometimes be observed (Fig. 12a). There are commonly circulating normoblasts and immature myeloid cells. The marrow is involved in a subtle and patchy fashion, but is sometimes extensively infiltrated with active hemophagocytosis seen (Fig. 12b). Immunophenotypically, these cases were typically CD2+, surface CD3- and CD56+. T-cell receptor gene is in germline configuration and EBV has been invariably demonstrated in the malignant cells (Imamura et al., 1990; Chan et al., 1997; Kwong et al., 1997a). In tissue specimens, the neoplastic infiltrate is diffuse, destructive and permeative. The lymphoid cell population often appears monomorphous, but can be polymorphous. The nuclei are often round, with fairly condensed chromatin. There is a thin to moderate rim of pale or amphophilic cytoplasm. Apoptotic bodies are frequently interspersed, and areas of zonal cell death are common. Angioinvasion and angiodestruction are

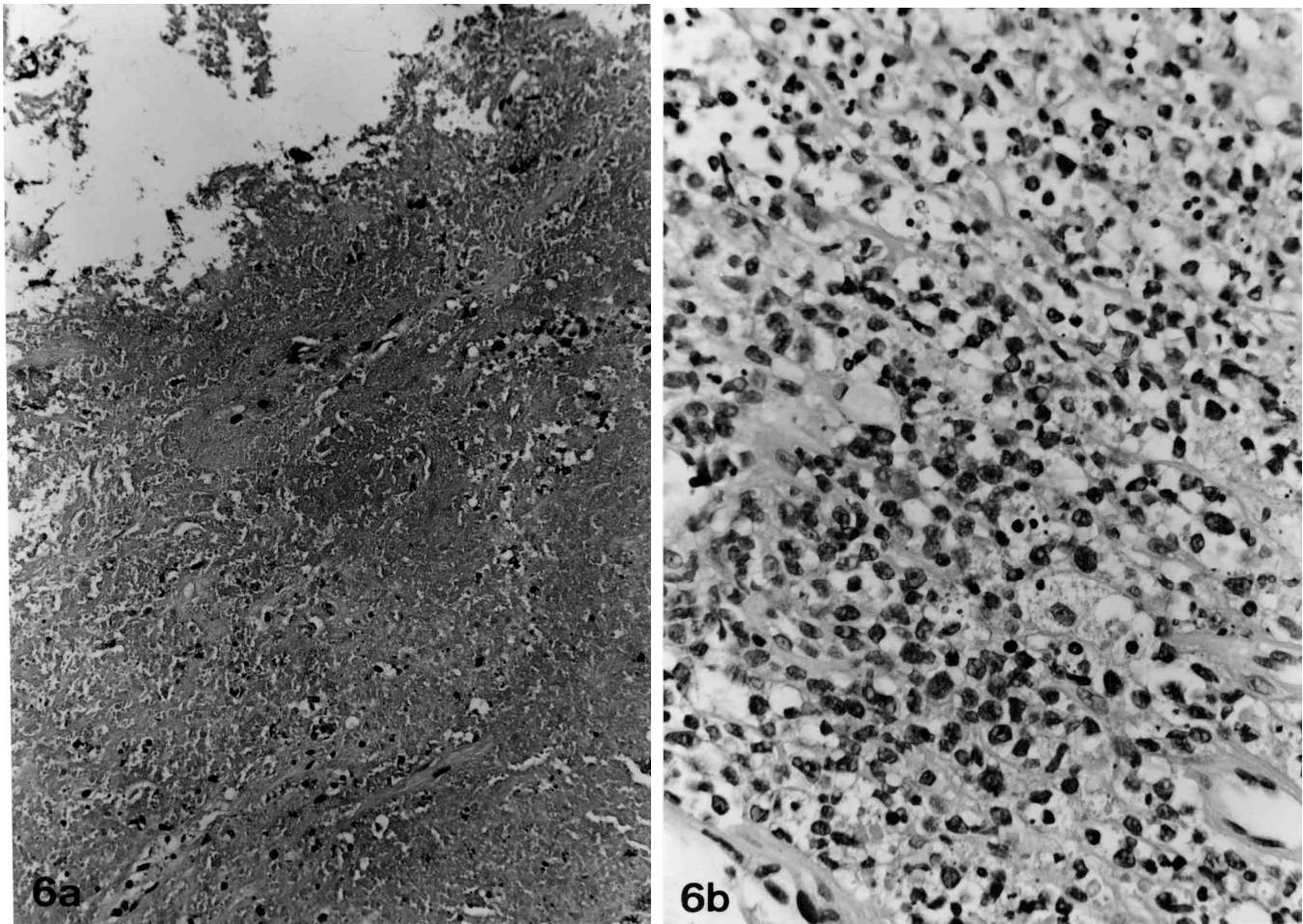


Fig. 6. Biopsy of nasal NK cell lymphoma. **a.** Frequently superficial biopsies yield necrotic tissue only, and are not diagnostic. x 100. **b.** Examination of the deeper portions of the biopsy or a new biopsy may reveal groups of lymphoma cells. x 100

Natural killer cell malignancies

common findings (Yatabe et al., 1994; Chan et al., 1997; Mori et al., 2000; Quintanilla-Martinez and Jaffe, 2000).

Post-transplantation lymphoproliferative disorder of NK cell lineage

Rare cases of post-transplantation lymphoproliferative disorder (PTLD) of NK cell lineage have been described (Hsi et al., 1998; Kwong et al., 2000; Mukai et al., 2000). All reported cases have so far occurred in renal allograft recipients, with a latency of 1 to 8 years post-transplantation. The morphologic, immunophenotypic and molecular features of these cases are similar to sporadic NK cell malignancies. EBV is demonstrable in two of the cases (Kwong et al., 2000; Mukai et al., 2000), but is absent from the third (Hsi et al., 1998). None of the cases responded to the withdrawal of immunosuppression. Whether these cases

represent chance occurrence of an NK cell malignancy in allograft recipients, or are genuine cases of PTLN remains to be defined by future studies.

Differential diagnosis

T cell lymphomas

The diagnostic hallmarks of T cell lymphoma are the frequent expression of surface CD3 and the presence of TCR gene rearrangements as demonstrated by Southern blot analysis. (Chan et al., 1995). If immunohistochemical studies are only carried out on paraffin sections, NK cell lymphomas and peripheral T cell lymphomas cannot always be differentiated, because NK cell lymphomas are cytoplasmic CD3+ and some T cell lymphomas are CD56+. True T cell lymphomas of the nasal area show less systemic dissemination and a

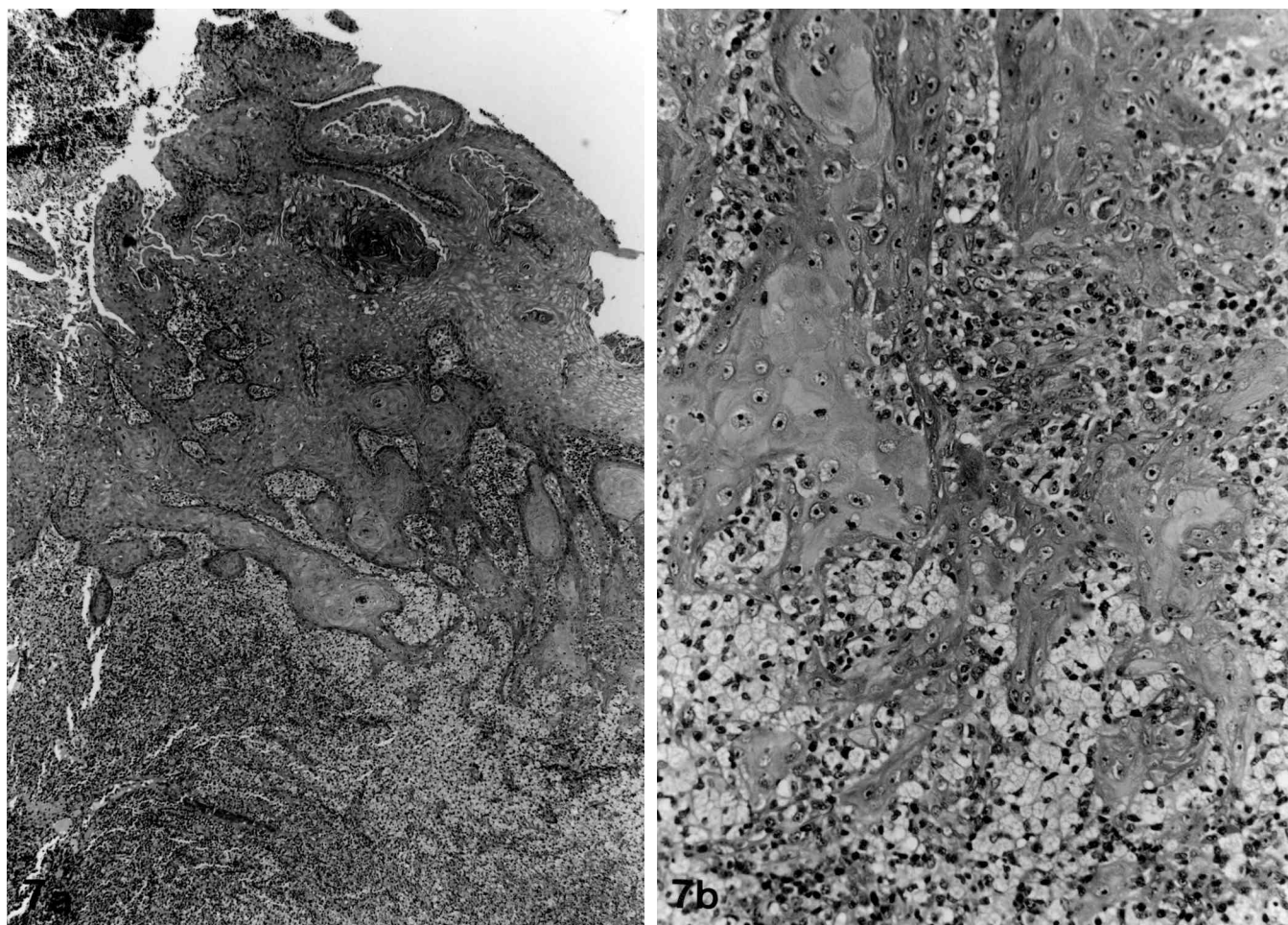


Fig. 7. Nasal NK cell lymphoma associated with florid pseudoepitheliomatous hyperplasia. **a.** Instead of ulceration, some nasal NK cell lymphomas are accompanied by striking hyperplasia of the overlying stratified squamous epithelium. x 50. **b.** The squamous epithelium can exhibit some degree of nuclear atypia, and can lead to a misdiagnosis of squamous cell carcinoma. The abnormal lymphoid cells between the squamous islands provide the clue to the correct diagnosis. x 150

slightly better outcome, although they are still considered a poor prognostic category of non-Hodgkin's lymphoma (Cheung et al., 1998). They are EBV positive in only a proportion of cases. The classification of occasional nasal lymphomas that are cytoplasmic CD3+, CD56-, cytotoxic markers+ and EBV+ is still controversial. Some studies include them as nasal NK/T cell lymphomas.

T large granular lymphocyte leukemia

T large granular lymphocyte leukemias (T-LGLL) are chronic indolent diseases marked by anemia, neutropenia, and recurrent infections (Loughran, 1993). Pure red cell aplasia complicates the disease in some patients (Kwong and Wong, 1998). T-LGLL differs from NK cell malignancies in that they are bona fide T cell malignancies with expression of T cell markers including CD3 and usually CD8, and have TCR gene

clonally rearranged. Occasionally, T-LGLL may also express CD56. In most cases, the neoplastic cells do not contain EBV.

Chronic NK cell lymphocytosis

Chronic NK cell lymphocytosis is a rare disorder that presents with a persistent increase in circulating NK cells that are CD3-CD16+CD56+ (Tefferi et al., 1994; Oshimi, 1996). The TCR gene should be in germline configuration. Clinically, chronic NK cell lymphocytosis is an indolent disease. Patients present with neutropenia, vasculitis and occasionally pure red cell aplasia. Because of the absence of a clonal marker, it is uncertain whether the condition is reactive or neoplastic.

Blastic NK cell lymphoma

Blastic NK cell lymphoma appears to be biologically

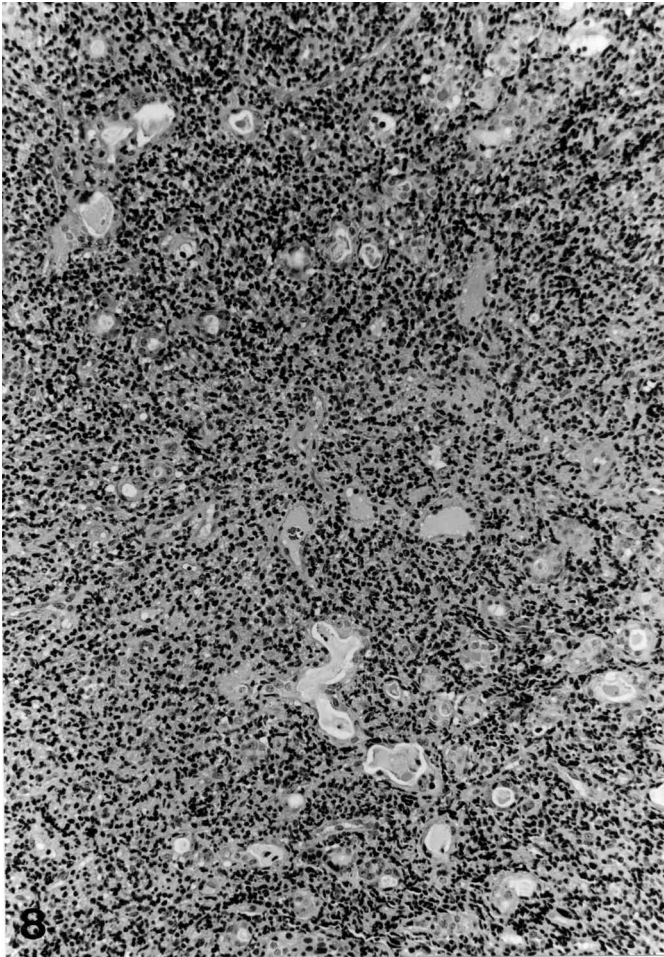


Fig. 8. Nasal NK cell lymphoma usually causes expansion of the respiratory mucosa, causing wide separation of the mucosal glands. x 50

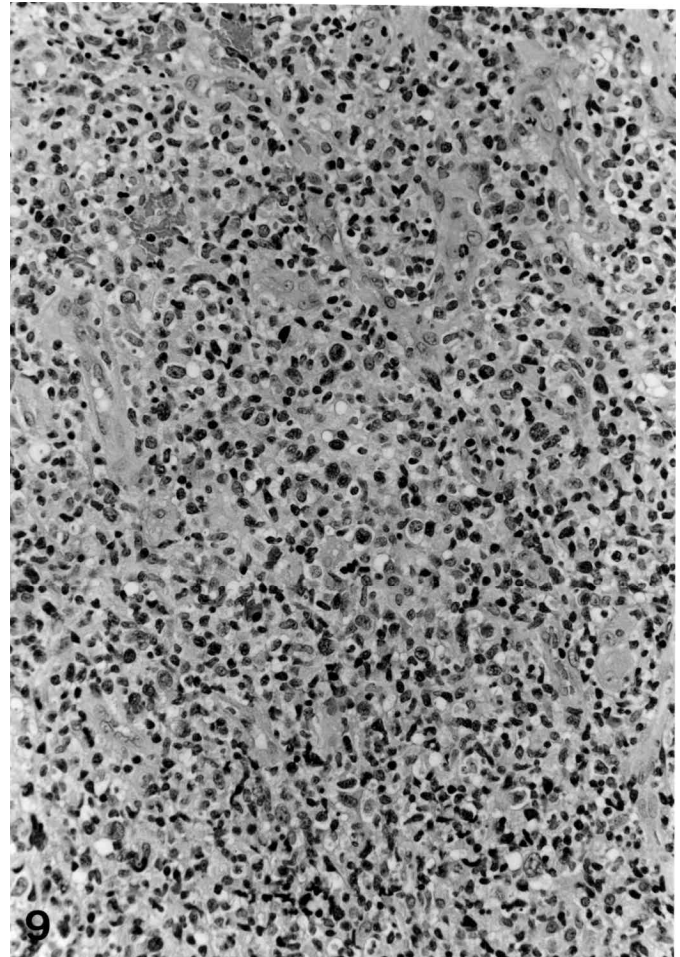


Fig. 9. Nasal NK cell lymphoma usually manifests as a diffuse dense lymphoid infiltrate that may appear polymorphic. x 150

Natural killer cell malignancies

distinct from other NK cell lymphoma/leukemia, in that the morphology is lymphoblastic or myeloblastic, there is variable expression of CD2, cytoplasmic CD3 and TdT, and EBV is not present (Nakamura et al., 1995; Kobashi et al., 1996). Patients present with extranodal disease that has a predilection for the skin. The skin lesions can be accompanied by lymph node or bone marrow involvement. Some cases may have a leukemic phase. The behaviour of this tumor remains to be clarified because only a few cases have ever been reported (Chan et al., 1997; DiGiuseppe et al., 1997).

Molecular pathology

Conventional cytogenetics analysis

Conventional karyotypic analysis of NK cell malignancies is difficult, partly owing to the necrosis

and scarcity of the clinical samples. However, several chromosomal abnormalities have been defined in a limited number of patients. Deletion of the long arm of chromosome 6, del(6q), is most frequently found, with the region 6q23 being commonly implicated (Tien et al., 1997; Wong et al., 1997). Chromosomal aberrations are restricted to the CD3-CD56+ tumor cell population, as demonstrated by concomitant surface immunophenotyping and fluorescence in situ hybridization (FISH) (Zhang et al., 1999). The finding of the same abnormality involving del(6q) in nasal and extranasal NK cell lymphoma as well as aggressive NK cell lymphoma/leukemia provides a biological link between these disease entities (Wong et al., 1997). Other non-random chromosomal abnormalities including +X, i(1q), i(7)(q10), +8, del(11q), del(13q), del(17)(p12) and i(17)(q10) have been reported (Wong et al., 1999). Although chromosomal translocations appear to be

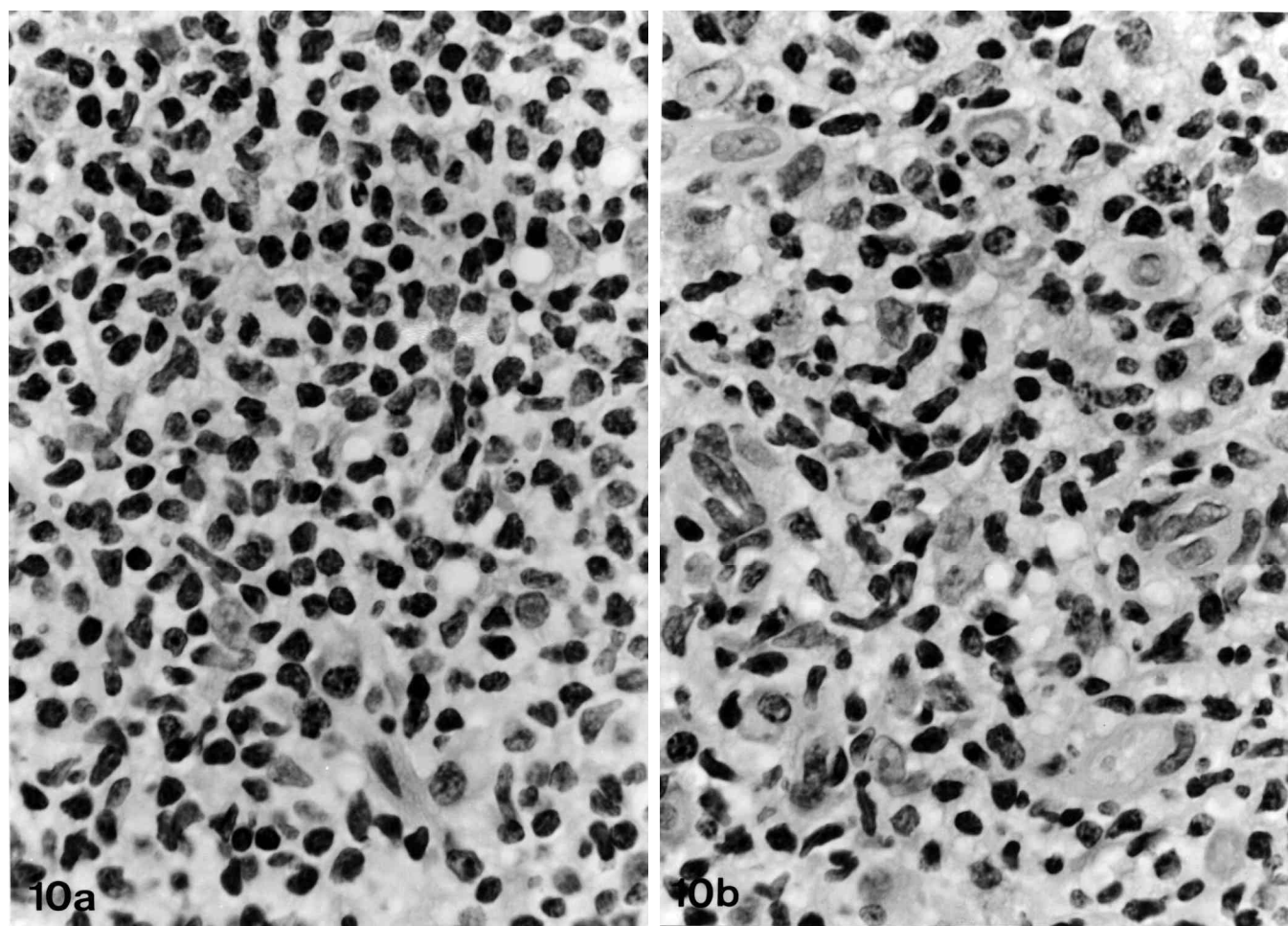


Fig. 10. Nasal NK cell lymphoma can exhibit a broad cytologic spectrum. **a.** An example composed of small cells with irregularly folded nuclei. Distinction from a reactive lymphoid infiltrate is very difficult. **b.** The small cells can have very elongated, serpentine nuclei. **c.** This example is composed of small to medium-sized cells with marked foldings of the nuclei. **d.** This example shows a mixture of small, medium-sized and large cells. **e.** An example comprising medium-sized to large cells. **f.** An example dominated by large cells. Many apoptotic bodies are seen – this is a common finding in NK cell lymphoma. x 400

uncommon, rearrangement involving chromosomal regions Xp21 and 8p23 have been described using spectral karyotyping (Wong et al., 2000).

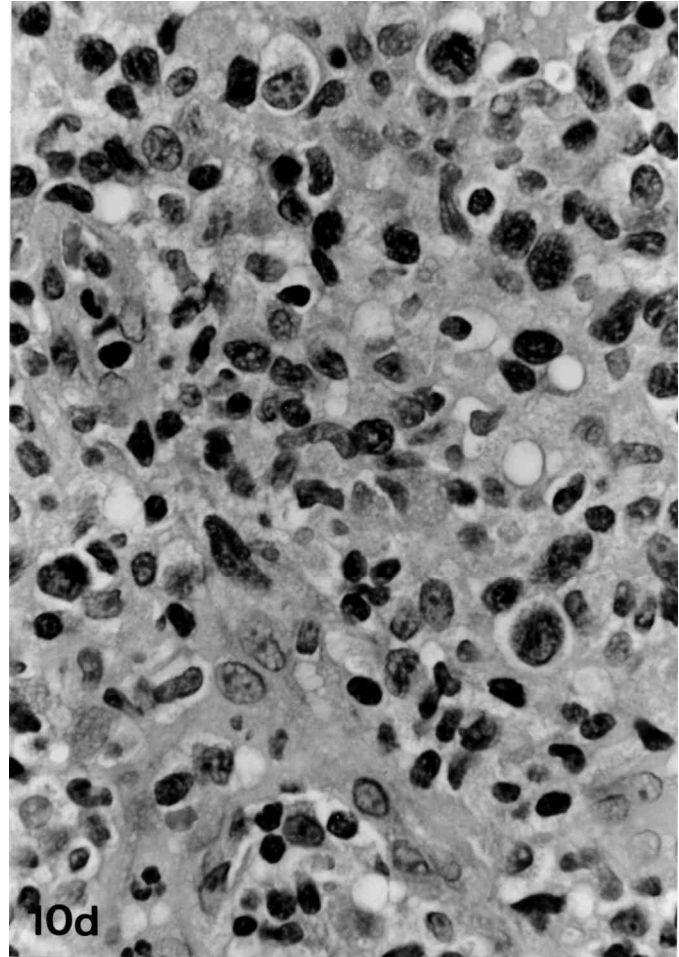
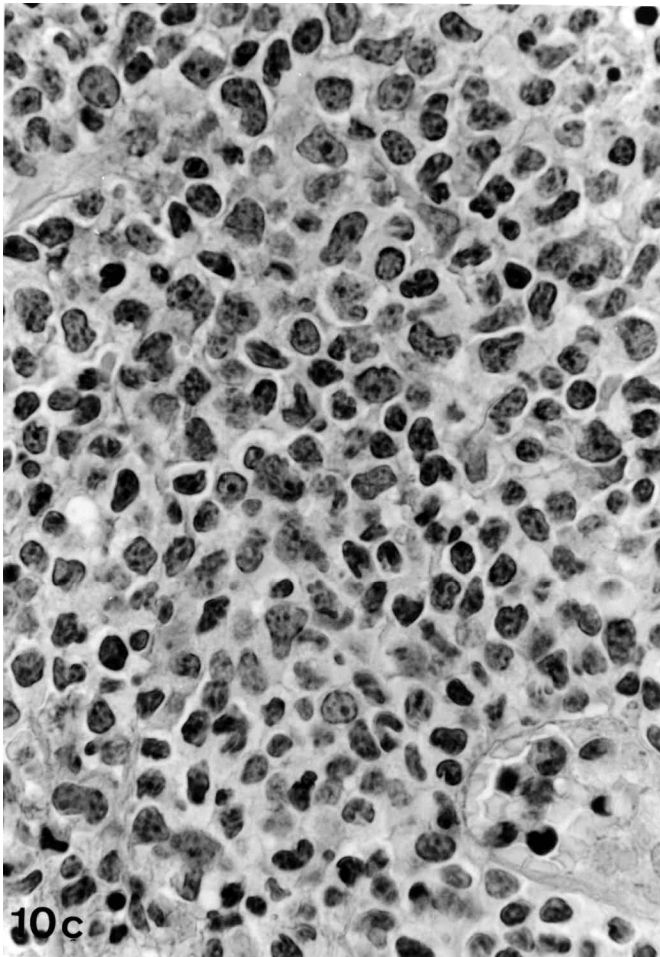
Comparative genomic hybridization

Comparative genomic hybridization (CGH) is a recently developed molecular cytogenetic technique that examines the entire genome of a tumor for chromosomal copy number aberrations in a single hybridization (Kallioniemi et al., 1992). CGH can be performed on DNA extracted from archival materials, and does not rely on the availability of fresh tissues or good-quality metaphases from the tumor clone. With CGH analysis (Siu et al., 1999), NK cell lymphomas demonstrate DNA losses in regions that correlate closely with conventional karyotyping, including del(6q), del(11q), del(13q), and del(17p). Compared with DNA losses, gains of DNA are more frequent and involve a large number of regions in the genome, including chromosomes 1p, 6p, 11q, 12q, 17q, 19p, 20q, and Xp. DNA gains are more frequently observed in nasal/extranasal lymphomas than in aggressive NK cell lymphoma/leukemia. On the other

hand, DNA gains in chromosome Xp are more frequent in aggressive NK cell lymphoma/leukemia. Regions of DNA losses and gains should be targets of investigation to identify putative tumor suppressor genes/proto-oncogenes (Siu et al., 1999).

Loss of heterozygosity analysis

The data from conventional karyotyping and CGH show that there is frequent DNA loss at chromosomes 6q, 11q, 13q and 17p. The pattern of DNA loss at these regions has been studied by loss of heterozygosity analysis (Siu et al., 2000). LOH in chromosomes 6q, 13q, 17p, and 11q occur with overall frequencies of 80%, 70.6%, 37.5%, and 33.3% respectively (Table 2). The pattern of LOH shows heterogeneity in different NK tumor subtypes and stage of the tumor. In nasal lymphoma, LOH at chromosome 13q is found in 33.3% of cases at presentation but in 100% of cases at relapse. This implies that 13q loss may be a progression event in nasal NK cell lymphoma. DNA loss at chromosomes 11q and 17p occurs at a lower frequency and is not detected in extranasal NK cell lymphoma. FISH analysis



Natural killer cell malignancies

shows that loss of 17p13 is more common in aggressive than nasal and extranasal NK cell lymphoma. The frequency of DNA loss at chromosome 13q is comparable with those of nasal and extranasal NK cell lymphoma.

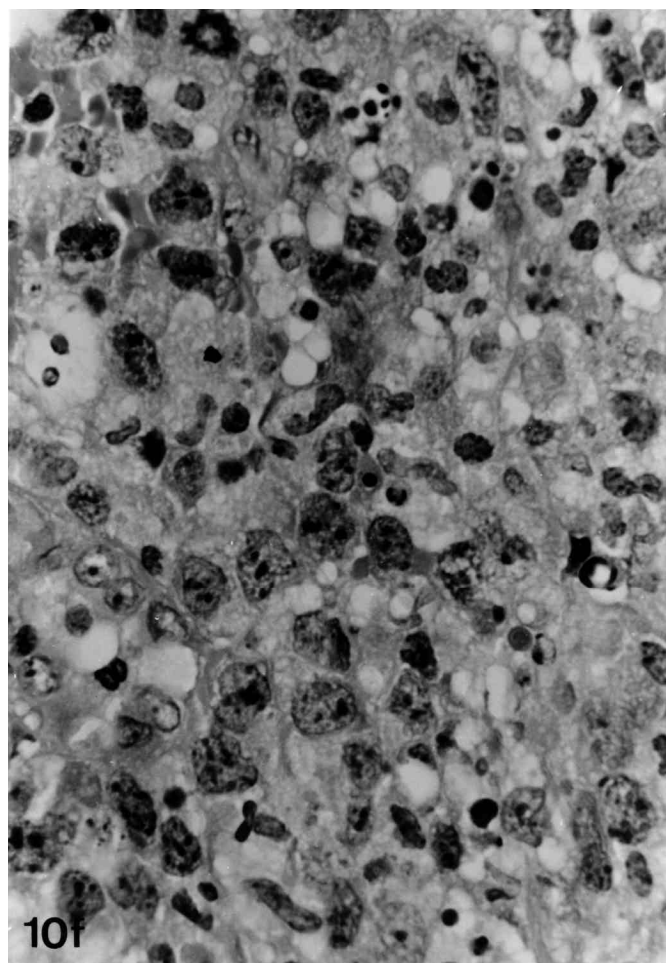
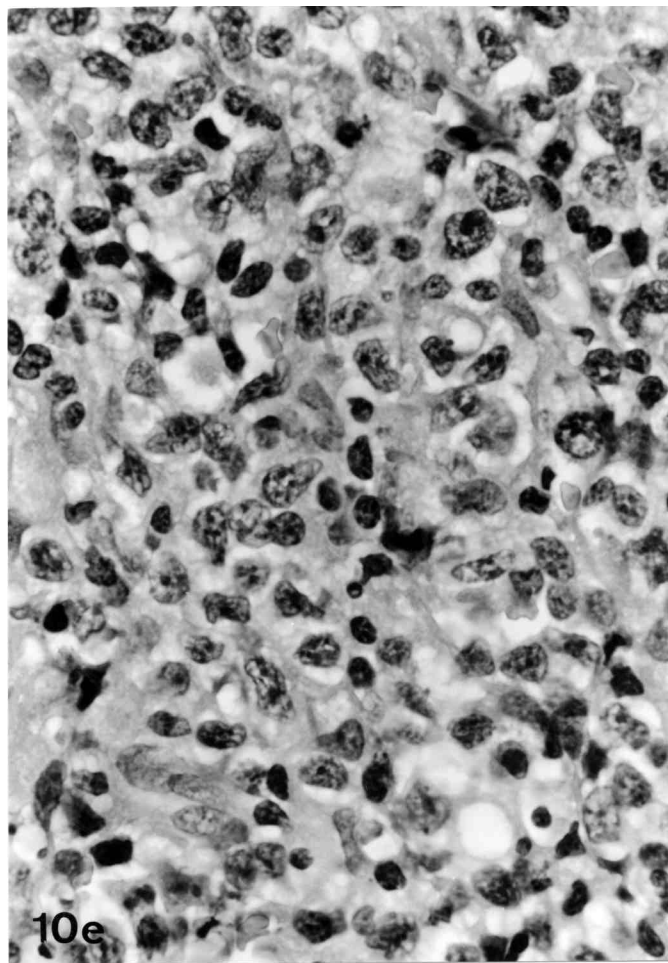
Association with Epstein Barr virus infection

The molecular pathogenesis initiating the malignant transformation of NK cells remains undefined. However, the almost invariable association with EBV infection

Table 2. Pattern of allelic loss based on clinical subtypes of natural killer cell malignancies.

	6q	11q	13q	17p
Commonly deleted region	6q13-q14 6q25	11q21-q24	13q12-q14 13q31-q34	17p12-p13
NK cell lymphoma	12/15 (80)	4/14 (28.6)	10/15 (66.7)	4/13 (30.8)
Nasal	10/11 (90.9)	4/10 (40)	7/11 (63.6)	4/9 (44.4)
At presentation	5/6 (83.3)	2/6 (33.3)	2/6 (33.3)	3/6 (50)
At relapse	5/5 (100)	2/4 (50)	5/5 (100)	1/3 (33.3)
Non-nasal	2/4 (50)	0/4 (0)	3 / 4 (75)	0/4 (0)
Aggressive NK cell lymphoma/leukemia	–	1/1 (100)	2/2 (100)	2/3 (66.7)
Total	12/15 (80)	5/15 (33.3)	12/17 (70.6)	6/16 (37.5)

No. of positive cases / No. of cases tested. Percentages are in parentheses.





suggests that it may play a role in tumor pathogenesis (Tao et al., 1995). Analysis of the terminal repeat region of the EBV genome shows that the virus is in a clonal episomal form. In addition to providing an indirect proof of the clonal nature of the lymphoid proliferation, it also implies that the EBV may play an etiologic role and is not merely a bystander (Medeiros et al., 1991; Minarovits et al., 1994; Kaneko et al., 1995; Kwong et al., 1997b). The selective strong association with EBV in NK cell lymphoma, but not T cell or B cell lymphomas of the nasal cavity provides additional evidence of the role of EBV in the pathogenesis of NK cell lymphomas (Chan et al., 1994). In the lymphoma cells, the EBV is present as a type II latency state, a pattern shared by Hodgkin's lymphoma and nasopharyngeal carcinoma. EBNA1 is expressed, EBNA2 to EBNA6 are not expressed, and there is a variable expression of latent membrane protein-1 (LMP1) (Kanavaros et al., 1993; Minarovits et al., 1994; van Gorp et al., 1996). The downregulation of the immunogenic EBV antigens EBNA2 - EBNA6 may help to explain how the tumor cells evade the immune surveillance by the host cytotoxic cells (Klein, 1994). Finally, EBV encoded RNAs (EBERs) which are not translated, are abundantly expressed in the majority of the neoplastic cells, and provide useful targets for the detection of EBV by ISH

Fig. 11. A common feature of nasal NK cell lymphoma is angioinvasion and angiodestruction. The wall of this blood vessel is heavily infiltrated by lymphoma cells. x 100

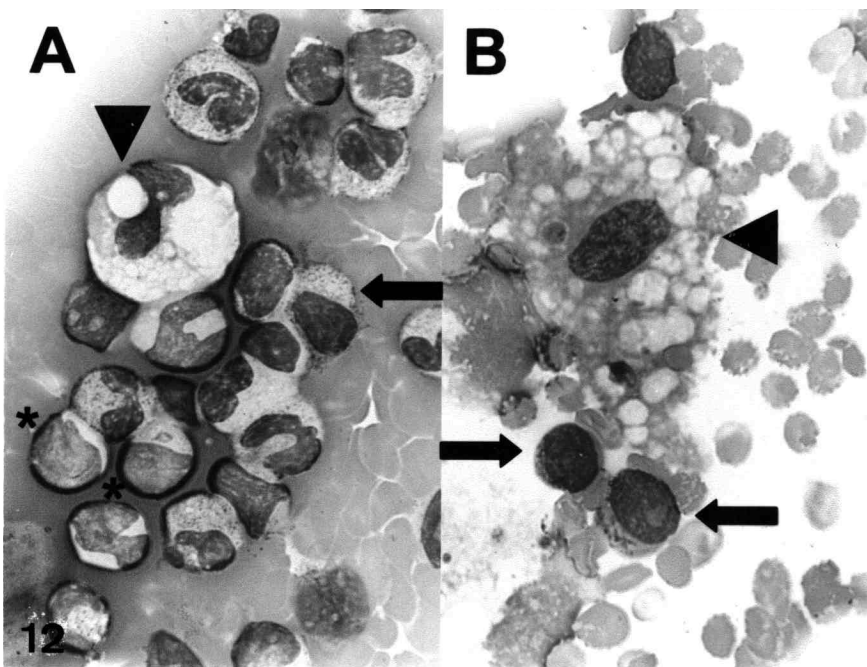


Fig. 12. A case of aggressive NK cell lymphoma/leukemia. **A.** Peripheral blood smear showing circulating NK lymphoma cells (arrow) with abundant cytoplasm containing azurophilic granules. Some tumor cells appear more blastic (asterisk). Hemophagocytosis (large arrow head) can sometimes be seen in the peripheral blood. **B.** Marrow aspirate showing NK lymphoma cell infiltration (arrows) associated with florid hemophagocytosis (large arrow head). x 1,000

techniques (Chan et al., 1994).

Geographical distribution of NK cell malignancies

NK cell malignancies are much more common in Orientals, Central Americans and South Americans than in Occidentals. Among about 200 cases of NK cell lymphomas reported in the literature, most are from Chinese, Japanese, Korean and South American patients, with only about 30 cases described in Western patients (Kanavaros et al., 1993; Macon et al., 1996). This dramatic geographical difference is intriguing. Even among sinonasal NK/T cell lymphomas seen in USA, most cases are not Caucasians (Gaal et al., 2000). This geographic difference may be explained in part by a difference in EBV epidemiology, as there is an almost invariable association of NK cell malignancies with EBV, which is much more prevalent in the Oriental people.

Treatment and prognosis

The clinical outcome of patients with nasal NK/T cell lymphoma is variable. Some patients are apparently cured by radiation therapy, while others show early local or systemic relapse, and succumb to the disease. Occasional patients even develop new disease within the irradiation field while receiving radiation therapy. The tumor may unpredictably disseminate to other sites, in particular the skin, liver, lymph node, gastrointestinal tract and testis, either early or late in the course. The results of chemotherapy with anthracycline-containing regimens have also been unsatisfactory (Kwong et al., 1997b). In several published series (Kwong et al., 1997a; Cheung et al., 1998), the median survival of these patients was about 12 months only.

Extranasal NK cell lymphomas are also clinically aggressive. Because the disease may be disseminated, chemotherapy is usually the initial choice of treatment. The response is poor, with most patients dying within 6 months of diagnosis (Kern et al., 1992; Chan et al., 1997).

Aggressive NK cell lymphoma/leukemia is a catastrophic disease with an almost uniform mortality. Patients usually present with a fulminant course marked by multi-organ failure, severe liver function derangement, and intravascular coagulopathy. The response to treatment is transient, and survival is measured in days to weeks. Allogeneic bone marrow transplantation in a case resulted only in short-term remission (Teshima et al., 1996). No reported survival of more than a year have been recorded (Imamura et al., 1990; Chan et al., 1997; Kwong et al., 1997b).

For patients with localized nasal lymphoma, high dose chemotherapy and autologous transplantation may result in improvement in survival (Liang et al., 1996). This mode of therapy is particularly suitable for localized NK cell lymphomas, where involvement of the bone marrow is rare (Kwong et al., 1997a; Wong et al.,

2001).

Conclusion

NK cell malignancies are aggressive neoplastic disorders with distinctive clinicopathologic features, molecular pathology, and geographical distribution. A consistent association with EBV infection suggests that the virus may be of pathogenetic significance. Further studies are needed to define the molecular pathogenesis and biologic markers that aid in the diagnosis and monitoring of treatment. Finally, clinical trials are needed to define the optimal therapeutic strategies that will lead to better survivals.

Acknowledgements. This study is supported by the Kadoorie Charitable Foundation.

References

- Aozasa K., Ohsawa M., Tomita Y., Tagawa S. and Yamamura T. (1995). Polymorphic reticulosis is a neoplasm of large granular lymphocytes with CD3+ phenotype. *Cancer* 75, 894-901.
- Chan J.K., Banks P.M., Cleary M.L., Delsol G., De Wolf-Peeters C., Falini B., Gatter K.C., Grogan T.M., Harris N.L., Isaacson P.G., Jaffe E.S., Knowles D.M., Mason D.Y., Muller-Hermelink H., Pileri S.A., Piris M.A., Ralfkiaer E., Stein H. and Warnke R.A. (1995). A revised European-American classification of lymphoid neoplasms proposed by the International Lymphoma Study Group: a summary version. *Am. J. Clin. Pathol.* 103, 543-560.
- Chan J.K.C. (1998). Natural killer cell neoplasms. *ASCP Rev. Pathol. (Anat. Pathol.)* 3, 77-145.
- Chan J.K.C., Sin V.C., Wong K.F., Ng C.S., Tsang W.Y.W., Chan C.H., Cheung M.M.C. and Lau W.H. (1997). Nonnasal lymphoma expressing the natural killer cell marker CD56: A clinicopathologic study of 49 cases of an uncommon aggressive neoplasm. *Blood* 89, 4501-4513.
- Chan J.K.C., Tsang W.Y.W. and Ng C.S. (1996). Clarification of CD3 immunoreactivity in nasal T/natural killer cell lymphomas: the neoplastic cells are often CD3e+. *Blood* 87, 839-840 (Letter).
- Chan J.K.C., Tsang W.Y.W., Ng C.S. and Pau M.Y. (1995). Discordant CD3 expression in lymphomas as studied on frozen and paraffin sections. *Human Pathol.* 26, 1139-1143.
- Chan J.K., Yip T.T., Tsang W.Y., Ng C.S., Lau W.H., Poon Y.F., Wong C.C. and Ma V.W. (1994). Detection of Epstein-Barr viral RNA in malignant lymphomas of the upper aerodigestive tract. *Am. J. Surg. Pathol.* 18, 938-946.
- Cheung M.M.C., Chan J.K.C., Foo W., Lau W.H., Chan P.T.M. and Ng C.S. (1998). Primary non-Hodgkin's lymphoma of the nose and nasopharynx: clinical features, tumor immunophenotype and treatment outcome in 113 patients. *J. Clin. Oncol.* 16, 70-77.
- Chiang A.K.S., Srivastava G., Lau P.W.F. and Ho F.C.S. (1996). Differences in T-cell receptor gene rearrangement and transcription in nasal lymphomas of NK and T-cell types: implications on cellular origin. *Human Pathol.* 27, 701-707.
- Davison S.P., Habermann T.M., Strickler J.G., DeRemee R.A., Earle J.D. and McDonald T.J. (1996). Nasal and nasopharyngeal lymphomas. *Laryngoscope* 106, 139-143.

Natural killer cell malignancies

- DiGiuseppe J.A., Louie D.C., Williams J.E., Miller D.T., Griffin C.A., Mann R.B. and Borowitz M.J. (1997). Blastic natural killer cell leukemia/lymphoma: a clinicopathologic study. *Am. J. Surg. Pathol.* 21, 1223-1230.
- Drenou B., Lamy T., Amiot L., Fardel O., Caulet-Maugendre S., Sasportes M., Diebold J., Le Prise P.Y. and Fauchet R. (1997). CD3- CD56+ non-Hodgkin's lymphomas with an aggressive behavior related to multidrug resistance. *Blood* 89, 2966-2974.
- Dukers D.F., Vermeer M.H., Jaspars L.H., Vos W., Willemze R. and Meijer C.J.L.M. (2001). 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. *J. Clin. Pathol.* 54, 224-228.
- Eichel B.S., Harrison E.G., Devine K.D., Scanlon P.W. and Brown H.A. (1966). Primary lymphoma of the nose including a relationship to lethal midline granuloma. *Am. J. Surg.* 112, 597-605.
- Emile J.F., Boulland M.L., Haioun C., Kanavaros P., Petrella T., Delfau-Larue M.H., Bensussan A., Farcet J.P. and Gaulard P. (1996). CD5-CD56+ T-cell receptor silent peripheral T-cell lymphomas are natural killer cell lymphomas. *Blood* 87, 1466-1473.
- Gaal K., Sun N.C., Hernandez A.M. and Arber D.A. (2000). Sinonasal NK/T-cell lymphomas in the United States. *Am. J. Surg. Pathol.* 24, 1511-1517.
- Haedicke W., Ho F.C., Chott A., Moretta L., Rudiger T., Ott G. and Muller-Hermelink H.K. (2000). Expression of CD94/NKG2A and killer immunoglobulin-like receptors in NK cells and a subset of extranodal cytotoxic T-cell lymphomas. *Blood* 95, 3628-3630.
- Harris N.L., Jaffe E.S., Stein H., Banks P.M., Chan J.K., Cleary M.L., Delsol G., De Wolf-Peeters C., Falini B. and Gatter K.C. (1994). A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 84, 1361-1392.
- Ho F.C., Choy D., Loke S.L., Kung I.T., Fu K.H., Liang R., Todd D. and Khoo R.K. (1990). Polymorphic reticulosis and conventional lymphomas of the nose and upper aerodigestive tract: a clinicopathologic study of 70 cases, and immunophenotypic studies of 16 cases. *Human Pathol.* 21, 1041-1050.
- Hsi E.D., Picken M.M. and Alkan S. (1998). Post-transplantation lymphoproliferative disorder of the NK cell type: a case report and review of the literature. *Mod. Pathol.* 11, 479-484.
- Imamura N., Kisunoki Y., Kajihara H., Okada K. and Kuramoto A. (1988). Aggressive natural killer cell leukemia/lymphoma with N901-positive surface phenotype: evidence for the existence of a third lineage in lymphoid cells. *Acta. Hematol.* 80, 121-128.
- Imamura N., Kusunoki Y., Kawa-Ha K., Yumura K., Hara J., Oda K., Abe K., Dohy H., Inada T., Kajihara H. and Kuramoto A. (1990). Aggressive natural killer cell leukaemia/lymphoma: report of four cases and review of the literature. Possible existence of a new clinical entity originating from the third lineage of lymphoid cells. *Br. J. Hematol.* 75, 49-59.
- Jaffe E.S., Chan J.K.C., Su I.J., Feller A.C., Frizzera G., Mori S. and Ho F.C.S. (1996). Report of the Workshop on nasal and related extranodal angiocentric T/natural killer cell lymphomas: definitions, differential diagnosis and epidemiology. *Am. J. Surg. Pathol.* 20, 103-111.
- Jaffe E.S., Harris N.L., Stein H. and Vardiman J.W. (2001). World Health Organization classification of Tumours. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. IARC Press. Lyon. pp 189-230.
- Kallioniemi A., Kallioniemi O.P., Sudar D., Rutovitz D., Gray J.W., Waldman F.M. and Pinkel D. (1992). Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258, 818-821.
- Kanavaros P., Lescs M.C., Briere J., Divine M., Galateau F., Joab I., Bosq J., Farcet J.P., Reyes F. and Gaulard P. (1993). Nasal T-cell lymphoma: a clinicopathologic entity associated with peculiar phenotype and with Epstein-Barr virus. *Blood* 81, 2688-2695.
- Kaneko T., Fukuda J. and Yoshihara T. (1995). Nasal NK cell lymphoma: report of a case with activated NK cells containing EBV and expressing CD21 antigen, and comparative studies of their phenotype and cytotoxicity with normal NK cells. *Br. J. Hematol.* 91, 355-361.
- Kassel S.H., Echevarria R.A. and Guzzo F.P. (1969). Midline malignant reticulosis (so called lethal midline granuloma). *Cancer* 23, 920-935.
- Kern W.F., Spier C.M., Hanneman E.H., Miller T.P., Matzner M. and Grogan T.M. (1992). Neural cell adhesion molecule-positive peripheral T-cell lymphoma: a rare variant with a propensity for unusual sites of involvement. *Blood* 79, 2432-2437.
- Kern W.F., Spier C.M., Miller T.P. and Grogan T.M. (1993). NCAM (CD56)-positive malignant lymphoma. *Leuk. Lymphoma* 12, 1-8.
- Klein G. (1994). Epstein-Barr virus strategy in normal and neoplastic B cells. *Cell* 77, 791-793.
- Kobashi Y., Nakamura S., Sasajima Y., Koshikawa T., Yatabe Y., Kitoh K., Mori S., Ueda R., Yamabe H. and Suchi T. (1996). Inconsistent association of EBV with CD56 (NCAM)-positive angiocentric lymphomas occurring in sites other than the upper and lower respiratory tract. *Histopathology* 28, 111-120.
- Kwong Y.L., Chan A.C.L., Liang R., Chiang A.K.S., Chim C.S. and Chan T.K. (1997a). CD56+ NK lymphomas: clinicopathologic features and prognosis. *Br. J. Haematol.* 97, 821-829.
- Kwong Y.L. and Wong K.F. (1998). High frequency of pure red cell aplasia in patients with T large granular lymphocyte leukemia. *J. Clin. Pathol.* 51:672-675.
- Kwong Y.L., Chan A.C.L. and Liang R.H. (1997b). Natural killer cell lymphoma/leukemia: pathology and treatment. *Hematol. Oncol.* 15, 71-79.
- Kwong Y.L., Lam C.C.K. and Chan T.M. (2000). Post – transplantation lymphoproliferative disease of natural killer cell lineage : a clinicopathological and molecular analysis. *Br. J. Haematol.* 110, 197-202.
- Lanier L.L., Chang C., Spits H. and Phillips J.H. (1992). Expression of cytoplasmic CD3 proteins in activated human adult natural killer (NK) cells and CD3 , , complexes in fetal NK cells. *J. Immunol.* 149, 1876-1880.
- Lanier L.L. (1998). NK cell receptors. *Annu. Rev. Immunol.* 16, 359-393.
- Liang R., Chen F., Lee C.K., Kwong Y.L., Chim C.S., Yau C.C. and Chiu E. (1996). Autologous bone marrow transplantation for primary nasal T/NK cell lymphoma. *Bone Marrow Transplantation* 19, 91-93.
- Loughran T.P. (1993). Clonal diseases of large granular lymphocytes. (Review) *Blood* 82, 1-14.
- Macon W.R., Williams M.E. and Greer J.P. (1996). Natural killer-like T cell lymphomas: aggressive lymphomas of T-large granular lymphocytes. *Blood* 87, 1474-1483.
- Medeiros L.J., Peiper S.C., Elwood L., Yano T., Raffeld M. and Jaffe E.S. (1991). Angiocentric immunoproliferative lesions: a molecular analysis of eight cases. *Human Pathol.* 22, 1150-1157.
- Minarovits J., Hu L.F., Imai S., Harabuchi Y., Kataura A., Minarovits-Kormuta S., Osato T. and Klein G. (1994). Clonality, expression and

Natural killer cell malignancies

- methylation patterns of the EBV genomes in lethal midline granulomas classified as peripheral angiocentric T-cell lymphomas. *J. Gen. Virol.* 75, 77-84.
- Moretta A. (1997). Molecular mechanisms in cell-mediated cytotoxicity. *Cell* 90, 13-18.
- Mori N., Yatabe Y., Oka K., Kinoshita T., Kobayashi T., Ono T. and Asai J. (1996). Expression of perforin in nasal lymphoma: additional evidence of its natural killer cell derivation. *Am. J. Pathol.* 149, 699-705.
- Mori N., Yamashita Y., Tsuzuki T., Nakayama A., Nakazawa M., Hasegawa Y., Kojima H. and Nagasawa T. (2000). Lymphomatous features of aggressive NK cell leukaemia/lymphoma with massive necrosis, haemophagocytosis and EB virus infection. *Histopathology* 37, 363-371.
- Mukai H.Y., Kojima H., Suzukawa K., Hori M., Komeno T., Hasegawa Y., Ninomiya H., Mori N. and Nagasawa T. (2000). Nasal natural killer cell lymphoma in a post-renal transplantation patient. *Transplantation* 69, 1501-1503.
- Nakamura S., Suchi T., Koshikawa T., Kitoh K., Koike K., Komatsu H., Iida S., Kagami Y., Ogura M., Katoh E., Kurita S., Suzuki H., Kobashi Y., Yamabe H., Hirabayashi N., Ueda R. and Takahashi T. (1995). Clinicopathologic study of CD56 (NCAM) positive angiocentric lymphoma occurring in sites other than the upper and lower respiratory tract. *Am. J. Surg. Pathol.* 19, 284-296.
- Ng C.S., Lo S.T.H., Chan J.K.C. and Chan W.C. (1997). CD56+ putative natural killer cell lymphomas: production of cytolytic effectors and related proteins mediating tumor cell apoptosis? *Human Pathol.* 28, 1276-1282.
- Noorduyn L.A., Torenbeek R., van der Valk P., Drosten P.B., Snow G.B., Balm A.J., Ossenkoppele G.J. and Meyer C.J. (1991). Sinonasal non-Hodgkin's lymphomas and Wegener's granulomatosis, a clinicopathologic study. *Virchows Arch. (A)* 418, 235-240.
- Ohno T., Kanoh T., Arita Y., Fujii H., Kuribayashi K., Masuda T., Horiguchi Y., Taniwaki M., Nosaka T., Hatanaka M. and Uchino H. (1988). Fulminant clonal expansion of large granular lymphocytes, characterization of their morphology, phenotype, genotype and function. *Cancer* 62, 1918-1927.
- Ohno Y., Amakawa R., Fukuhara S., Huang C.R., Kamesaki H., Amano H., Imanaka T., Takahashi Y., Arita Y., Uchiyama T., Kita K. and Miwa H. (1989). Acute transformation of chronic large granular lymphocyte leukemia associated with additional chromosomal abnormality. *Cancer* 64, 63-67.
- Okuda T., Sakamoto S., Deguchi T., Misawa S., Kashima K., Yoshihara T., Ikushima S., Hibi S. and Imashuku S. (1991). Hemophagocytic syndrome associated with aggressive natural killer cell leukemia. *Am. J. Hematol.* 38, 321-323.
- Oshimi K. (1996). Lymphoproliferative disorders of natural killer cells. *Int. J. Hematol.* 63, 279-290.
- Quintanilla-Martinez L. and Jaffe E.S. (2000). Aggressive NK cell lymphomas: insights into the spectrum of NK cell derived malignancies. *Histopathology* 37, 372-374.
- Robertson M.J. and Ritz J. (1990). Biology and clinical relevance of human NK cells. *Blood* 76, 2421-2438.
- Salmeron A., Sanchez-Madrid F., Ursa M.A., Fresno M. and Alarcon B. (1991). Conformational epitope expression upon association of CD3 with either CD3 or CD3 is the main target for recognition by anti-CD3 monoclonal antibodies. *J. Immunol.* 147, 3047-3052.
- Sanchez M.J., Spits H., Lanier L.L. and Phillips J.H. (1993). Human NK cell committed thymocytes and their relation to the T cell lineage. *J. Exp. Med.* 78, 1857-1866.
- Sanchez M.J., Muench M.O., Roncarolo M.G., Lanier L.L. and Phillips J.H. (1994). Identification of a common T/NK cell progenitor in human fetal thymus. *J. Exp. Med.* 180, 569-576.
- Soler J., Bordes R., Ortuno F., Montagud M., Martorell J., Pons C., Nomdedeu J., Lopez-Lopez J.J., Prat J. and Rutllant M. (1994). Aggressive natural killer cell leukaemia/lymphoma in two patients with lethal midline granuloma. *Br. J. Haematol.* 86, 659-662.
- Siu L.L.P., Chan V., Chan J.K.C., Wong K.F., Liang R. and Kwong Y.L. (2000). Consistent patterns of allelic loss in natural killer cell lymphoma. *Am. J. Pathol.* 157, 1803-1809.
- Siu L.L.P., Wong K.F., Chan J.K.C. and Kwong Y.L. (1999). Comparative genomic hybridization analysis of natural killer cell lymphoma/leukemia: recognition of consistent patterns of genetic alterations. *Am. J. Pathol.* 155, 1419-1425.
- Spits H., Lanier L.L. and Phillips J.H. (1995). Development of human T and natural killer cells. *Blood* 85, 2654-2670.
- Steward M., Bishop R., Piggott N.H., Milton I.D., Angus B. and Horne C.H. (1997). Production and characterization of a new monoclonal antibody effective in recognizing the CD3 T-cell associated antigen in formalin-fixed embedded tissue. *Histopathology* 30, 16-22.
- Stewart J.P. (1933). Progressive lethal granulomatous ulceration of the nose. *J. Laryngol. Otol.* 48, 657-701.
- Suzumiya J., Takeshita M., Kimura N., Kikuchi M., Uchida T., Hisano S., Eura Y., Kozuru M., Nomura Y. and Tomita K. (1994). Expression of adult and fetal NK cell markers in sinonasal lymphomas. *Blood* 83, 2255-2260.
- Tao Q., Ho F.C.S., Loke S.L. and Srivastava G. (1995). Epstein-Barr virus is localized in the tumor cells of nasal lymphomas of NK, T or B cell types. *Int. J. Cancer* 60, 315-320.
- Tefferi A., Li C.Y., Witzig T.E., Dhodapkar M.V., Okuno S.H. and Phyliky R.L. (1994). Chronic NK cell lymphocytosis: a descriptive clinical entity. *Blood* 84, 2721-2725.
- Teshima T., Miyaji R., Fukuda M. and Ohshima K. (1996). Bone marrow transplantation for Epstein-Barr virus-associated natural killer cell-large granular lymphocyte leukemia. *Lancet* 347, 1124 (Letter).
- Tien H., Su I., Tang J., Liu M., Lee F., Chen Y. and Chuang S. (1997). Clonal chromosomal abnormalities as direct evidence for clonality in nasal T/natural killer cell lymphoma. *Br. J. Haematol.* 97, 621-625.
- van Gorp J., Brink A., Oudejans J.J., van den Brule A.J., van den Tweel J.G., Jiwa N.M., de Bruin P.C. and Meijer C.J. (1996). Expression of Epstein-Barr virus encoded latent genes in nasal T cell lymphomas. *J. Clin. Pathol.* 49, 72-76.
- Weiss L.M., Arber D.A. and Strickler J.G. (1994). Nasal T-cell lymphoma. *Ann. Oncol.* 5 (Suppl 1), S39-S42.
- Wong K.F., Chan J.K.C., Ng C.S., Lee K.C., Tsang W.Y.W. and Cheung M.M.C. (1992). CD56 (NKH1)-positive hematolymphoid malignancies: an aggressive neoplasm featuring frequent cutaneous/mucosal involvement, cytoplasmic azurophilic granules and angiocentricity. *Human Pathol.* 23, 798-804.
- Wong K.F., Chan J.K.C. and Kwong Y.L. (1997). Identification of del(6)(q21q25) as a recurring chromosomal abnormality of putative NK cell lymphoma/leukaemia. *Br. J. Haematol.* 98, 922-926.
- Wong K.F., Zhang Y. and Chan J.K.C. (1999). Cytogenetic abnormalities in natural killer cell lymphoma/leukaemia: is there a consistent pattern? (Invited Review). *Leuk. Lymphoma* 34, 241-250.
- Wong K.F., Chan J.K., Cheung M.M. and So J.C. (2001). Bone marrow involvement by nasal NK cell lymphoma at diagnosis is uncommon.

Natural killer cell malignancies

- Am. J. Clin. Pathol. 115, 266-70.
- Wong N., Wong K.F., Chan J.K. and Johnson P.J. (2000). Chromosomal translocations are common in natural killer-cell lymphoma/leukemia as shown by spectral karyotyping. *Human Pathol.* 31, 771-774.
- Yatabe Y., Mori N., Hirabayashi N. and Asai J. (1994). Natural killer cell leukemia, an autopsy case. *Arch. Pathol. Lab. Med.* 118, 1201-1204.
- Ye Y.L., Zhou M.H., Lu X.Y., Dai Y.R. and Wu W.X. (1992). Nasopharyngeal and nasal malignant lymphoma: a clinicopathological study of 54 cases. *Histopathology* 20, 511-516.
- Zhang Y., Wong K.F., Siebert R., Matthiesen P., Harder S., Eimermacher H., Feller A.C. and Schlegelberger B. (1999). Chromosome aberrations are restricted to the CD56+ CD3- tumour cell population in natural killer cell lymphomas: a combined immunophenotyping and FISH study. *Br. J. Haematol.* 105, 737-742.

Accepted December 7, 2001