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Invited Lecture

The Significance of Cytokine Expression in Hodgkin's Disease and Anaplastic Large Cell Lymphoma

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Cytokines are known to be involved in cellular proliferation and differentiation. Under the influence of these growth factors, cells having the equivalent receptor are driven into various differentiations forms from immature precursors to functional endstage cells. The cytokine network is a well balanced system, within which the different peptides can act in a hormone-like manner, in paracrine pathways as well as with autocrine mechanisms.

It is known that some clinical symptomes of reactive as well as of neoplastic diseases are due to an imbalance of a cytokine expression. Such factors may thus also influence the microenvironment in some neoplastic diseases. It is well documented that the eosinophilia in Hodgkin's disease is due to a secretion of IL-5 produced by the Hodgkin cells themselves[1]. The influence of tumor growth by cytokines has been shown for plasmocytomas[2]. The neoplastic cell growth can be induced by IL-6 either in a paracrine or autocrine pathway. In that cases IL-6 can be produced by either the neoplastic cell clone or by stromal cells. The specificity of the IL-6 effect can be demonstrated by blocking the tumor cell growth by specific anti-IL-6 antibodies[3].

The morphology of some malignant lymphomas in terms of cellular constituents or growth pattern clearly give indications for an interaction between neoplastic and non-neoplastic cell compartments. Some entities are characterized by a specific milieu of bystander cells (Hodgkin's disease) or a distinct growth pattern (e. g. centroblastic centrocytic lymphoma, T-zone lymphoma, T-cell lymphoma of AILD-type, anaplastic large cell lymphoma)[4,5].

Semiquantitative analysis of cytokine RNA of malignant lymphomas strongly indicates in some cases a deregulation of the cytokine expression. About one third of the cases show a strong overexpression of various cytokines at the Northern blot level, indicating an abundant quantity of these factors which are obviously produced by the tumor cells[6, 7, 8]. It must be additionally kept in mind that cytokines physiologically act at extremely low concentrations. However, the expression in malignant lymphomas is completely unpredictable which means that a distinct lymphoma entity does not exhibit a distinct cytokine profile.

A recently described T cell growth factor - interleukin 9[9]-has been shown to have the typical pleiotropic effect on different cell lineages. Among these effects IL-9 simulates the proliferation of mature T cells. Clonally amplified T cells survive in vitro under the influence of exogenous Il-9 indefinitely. Interestingly, IL-9 expression is within the lymphoid cell system restricted to stimulated peripheral T lymphocytes and to the lymphoma entites anaplastic large cell lymphoma and Hodgkin's disease by means of Northern blot analysis (table 1) as well as by PCR. Other B - and T cell lymphomas are negative[7].

Table 1. Cytokine expression in Hodgkin's disease (HD), anaplastic large cell lymphoma (LCAL) and lymphoblastoid cell lines (LBC).
Different cytokines are expressed in the different lymphoma types. IL-9 expression is restricted to these three entities.

	n	IL-2	IL-4	IL-6	IL-7	IL-9	IL-10
HD	(16)	-	3	11	7/7	7	8
LCAL	(8)	-	1	5	n.d.	4	n.d.
LBC	(5)	2	5	5	5	4	5

Within the mouse system IL-9-dependent CD4-positive T cells can be transfected with recombinant IL-9 cDNA which results in an autocrine production of IL-9 and an independent clonal T-cell growth. The injection of these transfected T-cell clones into mice results in the development of a malignant lymphoma in all animals. Non-transfected exogeneous IL-9-dependent T-cell clones never induced a tumor growth when

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injected into mice[9,10].

The morphological investigation of the animal tumors revealed after injection into the peritoneal cavity the spread of a malignant lymphoma. All animals showed a lymph node enlargement in the abdomen. The subcutaneous application of the IL-9 autocrine T-cell clones resulted in evolvement of skin tumors. Morphologically the tumors constituted from large bizarre anaplastic cells, some of which strongly resembled Hodgkin or Sternberg-Reed cells. Some tumors showed a cohesive growth pattern and were accompanied by an eosinophilia (fig.1-3). Moreover, early involvement of lymph nodes exhibited an intrasinusoidal growth pattern. The tumor infitrates showed a diffuse fibrosis of the lymphnode tissue. The immunophenotypic data revealed an expression of CD4 and CD25 on the tumor cells. The T cell receptor B-chain gene showed a clonal rearrangement pattern[10].

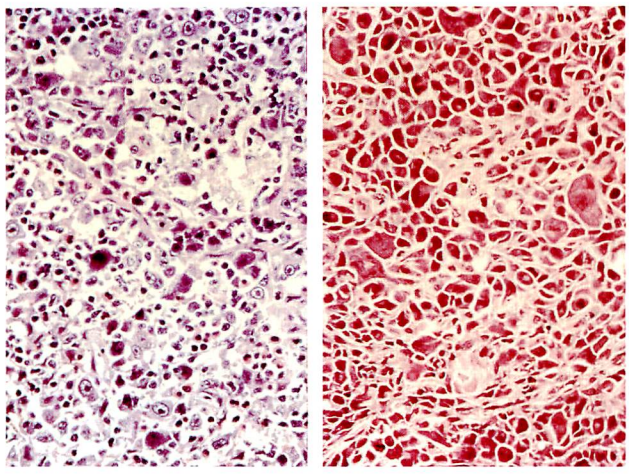


Fig.1. A comparison between Hodgkin's disease, lymphocyte depletion compared to the anaplastic mouse tumor (right side) showing large bizarre blasts resembling Hodgkin and Sternberg-Reed cells. Additionally a cohesive growth pattern is visible.

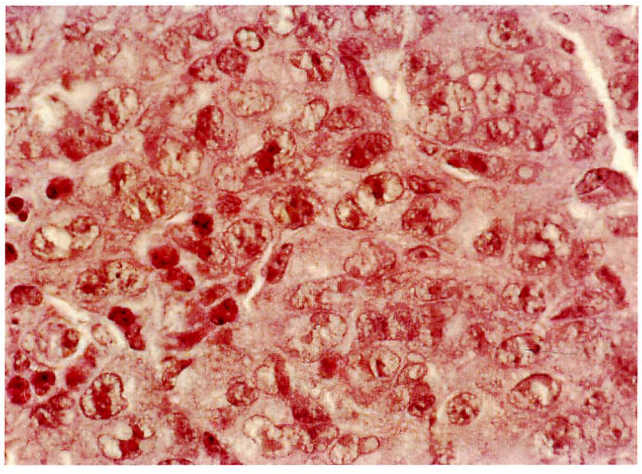


Fig.2. High power view of the mouse tumor cells with large anaplastic cells.

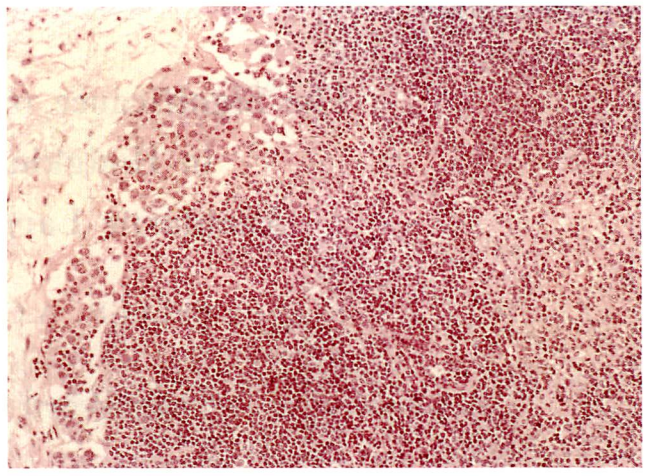


Fig.3. Intrasinusoidal growth pattern of murine anaplastic large cell lymphoma (upper and lower left).

Table 2. Comparison of morphological immunophenotypic and genotypic data between the mouse tumor evolving from autocrine mouse T-cells and human anaplastic large cell lymphoma expressing IL-9 and IL-9-receptors.

	IL-9 mouse tumor	LCAL human
TcRβ rec.	+	+
IL-2 R	+	+
CD71	+	+
CD30	?	+
eosinophilia	+	+
Hodgkin cells	+	+
intrasinusoidal tumor growth	+	+
T-zone infiltration	+	+
fibrosis	+	+

In table 2 the many similarities between human anaplastic large cell lymphomas expressing IL-9 and mouse T-cell lymphoma having Il-9 as an autocrine growth factor are given. Thus, it seems possible that IL-9 is involved in tumor proliferation and even in transformational events in human anaplastic large cell lymphoma. This is underlined by the observation that IL-9-RNA can be detected in Hodgkin and Sternberg-Reed cells in Hodgkin's disease by in situ hybridization[7].

Recently we were able to detect the translocation t (2;5) in cases of Hodgkin's disease[11], which, so far, was thought to be restricted to cases of anaplastic large cell lymphoma. This observation seems especially interesting under the aspect that the Il-9-gene is now described in its chromosomal location. the gene is located on the long arm of chromosome 5q32-q35. The translocation involves a locus on the long arm of chromosome at 5q35[12]. Thus, it might be possible that the chromosomal translocation is causing directly or indirectly via inducing transcription factors which might lead to a deregulation of IL-9 gene expression.

However, it seems highly probable that not only a

single cytokine or cytokine receptor is involved in tumor proliferation or transformational events but a variety of these ligands and their receptors may be of influence. In vitro experiments as well as in vivo studies indicate that the cytokine secretion is increased after cell stimulation. This is best known from PHA stimulated peripheral blood T lymphocytes. B cells however do not show an equivalent variety of cytokine expression after in vitro stimulation. EBV infection of B cells on the other hand leads to an over expression of activation antigens on the cell surface.

It has been shown that EBV-genome and protein can frequently be detected in some lymphoma types of B and T-cell orign including Hodgkin's disease and anaplastic large cell lymphoma which clearly show signs of cellular activation[13]. This observation raises the question on the significance of this EBV infection. The relevance is still unclear as most lymphoma entities which have been shown to carry EBV have detectable EBV genomes in only about 20 to 50% of the cases.

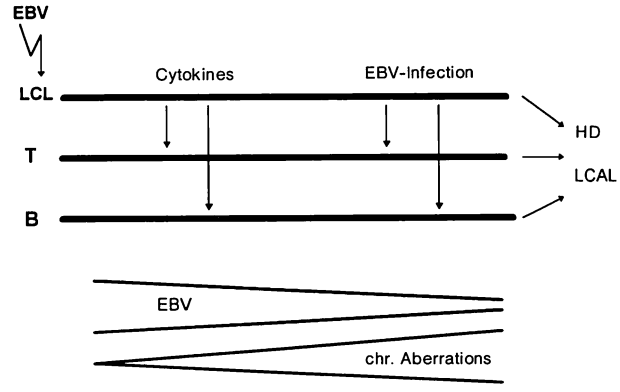
The in vitro prototype of EBV infected cells are the lymphoblastoid cells from which cell lines can easily be established from patients with EBV positive lymphomas or even from peripheral blood of normal human individuals having passed an EBV infection.

We have recently studied lymphoblastoid cell lines (EBV-infected B cells) in order to investigate the cellular events under the condition of longterm cell culture. In principle three major observations can be made:

1. The phenotype of the cells is variable over a time period with an increase of CD30 expression. In part also a CD15 expression is detectable.
2. The number of chromosomal abnormalities during a culture period of several months increases. Moreover an involvement of the long arm of chromosome 6 was observed, an abnormality which is frequently detected in Hodgkin's disease[14].
3. Lymphoblastoid B-cell lines express a number of cytokines at least on the level of mRNA which have been thought so far to be either T-cell derived factors or be produced only by stroma cells. This latter observation is of importance, as even a maximal antigenic stimulation of peripheral blood B lymphocytes is never able to induce such an amount of cytokine expression. Thus, this expression is due to the EBV-infection.

These experiments may give some more indications for the involvement of cytokines in some lymphoma types. The induction of cytokine expression or even overexpression induces cellular proliferation. The variety of cytokines may be able to act on different cell lineages, e.g. T cells, B cells, monocytes/histiocytes. An increasing and continuing cellular proliferation also increases the number of chromosomal aberrations which finally may lead to autonomous growth (table 3).

Table 3. A hypothetic scheme showing the possible involvement of EBV in lymphoma genesis. EBV infection leads to an increse of cytokine expression which may act on different cellular lineages or may even infect different lymphoid cells. These phenomena are paralleled by an increase of chromosomal aberrations. A decrease of EBV expression at the proteine level is possible.



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