Hodgkin's disease: pursuing the progenitor cell

Chapter 7

Summary and General Discussion

Summary

This thesis describes the phenotyping and genotyping of both Hodgkin/Reed-Sternberg cells as well as morphologically normal cells in Hodgkin's disease. It is generally accepted that Hodgkin/Reed-Sternberg cells are part of the malignant cell fraction in Hodgkin's disease, but they constitute only a minor fraction of cells in the tumor [3, 15]. Hodgkin/Reed-Sternberg cells have been extensively analyzed to determine their origin and nature. Recently it has been concluded that the Hodgkin/Reed-Sternberg cells are derived from B-lymphocytes and that they originate from follicle center cells of the lymph node [1, 5, 6, 12, 13, 16, 22-26, 30].

We hypothesized that the malignant clone in Hodgkin's disease not only consists of Hodgkin/Reed-Sternberg cells, but also includes morphologically normal cells, which may be their progenitor cells. Flow cytometric analysis of Hodgkin's disease showed that the aneuploid cell fraction exceeded the Hodgkin/Reed-Sternberg cell population, indicating that other cells should also contain an aberrant genetic constitution [7]. Therefore, morphologically normal cells were investigated in this study in an attempt to identify precursor cells of the Hodgkin/Reed-Sternberg cells amongst this bystander cell population.

For the analysis of the phenotype and genotype of cells a novel triple-color detection procedure for brightfield microscopy has been developed (chapter 2) and was used to determine the phenotype and genetic constitution of both Hodgkin/Reed-Sternberg cells and morphologically normal cells (chapter 5). The procedure allowed a combined detection of phenotypic and genetic parameters. It was demonstrated for this purpose that two peroxidase detection reactions could be performed using different precipitating reagents, such as diaminobenzidine (brown) and tetramethylbenzidine (green). A mild acid treatment was used as an inactivation step between the two peroxidase detection reactions. In chapter 5, the two peroxidase enzyme reactions described in this procedure were used to determine CD30 and chromosome constitution of cells in Hodgkin's disease.

The phenotypic constitution of Hodgkin/Reed-Sternberg cells and morphologically normal cells has been analyzed and compared to determine whether certain characteristics may distinguish precursor cells in Hodgkin's disease and link them to Hodgkin/Reed-Sternberg cells. For this purpose lamin subtype and Ki-67 antigen expression have been analyzed in reactive lymph nodes and in Hodgkin's disease (chapter 3). It is shown that Hodgkin/Reed-Sternberg cells express A-type lamins, which indicates that these tumor cells are more differentiated than the morphologically normal cells that mostly lack A-type lamins. In general the expression of A-type lamins coincided with the absence of the cell proliferation marker Ki-67, although a small fraction of apparently differentiated (lamin A positive) Hodgkin/Reed-Sternberg cells did show reactivity with the Ki-67 antibody. Furthermore,

lamin B2 is present in Hodgkin/Reed-Sternberg cells but also in most bystander cells, with the exception of the follicle center cells of the reactive lymph node. Although differences in phenotypic constitution between Hodgkin/Reed-Sternberg cells and the morphologically normal cells exist, they in themselves were not able to recognize precursor cells in Hodgkin's disease.

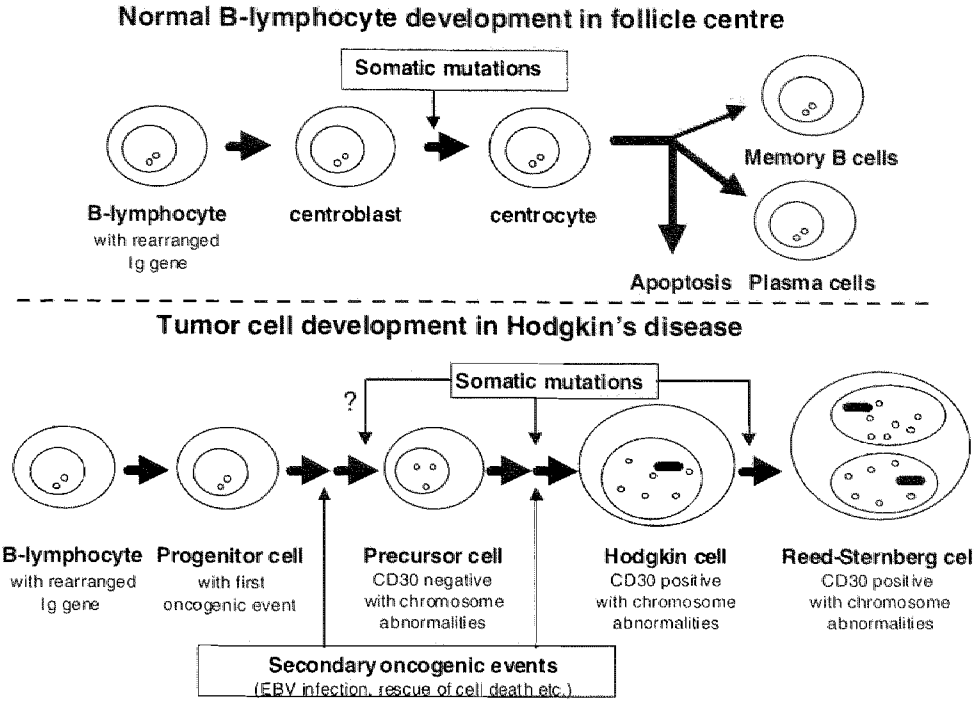
The genetic constitution of Hodgkin/Reed-Sternberg cells and morphologically normal cells was analyzed to see whether or not aneuploidy is restricted to the Hodgkin/Reed-Sternberg cells. Chapters 4 and 5 describe the results obtained with the in situ hybridization technique applied on Hodgkin's disease. In chapter 4, it has been demonstrated in six cases that not only Hodgkin/Reed-Sternberg cells but also very small populations of morphologically normal cells gain numerical chromosome abnormalities. The phenotypic constitution of these genetically aberrant, morphologically normal cells has been extensively explored in two cases (chapter 5), containing a relatively high fraction of such cells, showing that they express the CD19-antigen and lack the CD30-antigen. This indicates that the genetically aberrant, morphologically normal cells are of a B-lymphocyte origin. Results of chromosome analyses suggest that they are precursor cells of the Hodgkin/Reed-Sternberg cells, which gain additional chromosomal abnormalities during the development into Hodgkin/Reed-Sternberg cells.

In chapter 6 phenotypic and genetic characteristics of Hodgkin/Reed-Sternberg cells and of morphologically normal cells observed in the previous studies (chapters 3-5) have been analyzed in a cell line derived from a patient with Hodgkin's disease and compared to the original tumor. It was demonstrated that the expression of A-type lamins was increased in Hodgkin/Reed-Sternberg-like cells whereas small cells showed no increase. This suggests that the large Hodgkin/Reed-Sternberg-like cells are more differentiated than the small cells. The lack of CD30 antigen in the majority of small and large cells of the cell line, and the chromosomal abnormalities observed in these cells suggest that the cell line contain cell populations also observed in the tumor. Furthermore, the genotype of Reed-Sternberg-like cells in the cell line as compared to that of small cells, indicates that the large cells result from endoreduplication and not from cell fusion.

General discussion

Reflecting upon the data presented in this thesis, it can be stated that an important piece has been added to the complicated jigsaw puzzle called Hodgkin's disease. The technique we used in this quest was a combined immunocytochemistry and in situ hybridization procedure. This combined procedure allowed us to identify a precursor cell population in Hodgkin's disease. These precursor cells have been characterized as CD30 negative B-lymphocytes, and their malignant nature is demonstrated by their chromosomal abnormalities.

The value of this work lies in the identification of these precursor cells and in describing the in situ hybridization procedure to identify them. However, the identification of the precursor cells is only at its beginning. These cells have to be characterized phenotypically and genotypically in more detail and the position of the precursor cells in the sequence of events involved in tumor cell development has to be established for Hodgkin's disease (see figure) [27, 35].



From the identification of immunoglobulin gene rearrangements in Hodgkin/Reed-Sternberg cells it may be inferred that the progenitor and precursor cells are derived from follicle centre cells. At some point the precursor cells transform, acquiring the morphology of a Hodgkin cell, including the expression of CD30. This transformation event may perhaps be compared with the development of a centroblast out of a naïve lymphocyte in the follicle centre upon activation by antigen (see figure). Indeed cells with the morphology of Hodgkin cells and even of Reed-Sternberg cells may be found in normal lymph nodes upon activation. The comparison with the events in a normal follicle centre ends at this point, since centrocytes are not formed out of Hodgkin cells but instead multinucleated Reed-Sternberg cells emerge, perhaps through endoreduplication.

Analysis of tumor cell development in Hodgkin's disease, however, may be performed further against the background of the dynamics in the follicle centres [19, 28]. From this point of view, the role of activation by antigen has to be addressed as well as the role of T-cell help, next to B-cell maturation. Precursor cells, therefore, have to be characterized for phenotypic and genetic markers in order to obtain a proper understanding of the molecular mechanisms involved, for example, in activation, in differentiation and in programmed cell death rescue.

If activation by antigen would be a critical factor in lymphocyte development, the question remains which antigen in Hodgkin's disease is the critical factor for the transformation of the precursor cell into a Hodgkin/Reed-Sternberg cell? Epstein-Barr virus related antigens might be good candidates, because Hodgkin/Reed-Sternberg cells of classical Hodgkin's disease are often infected with Epstein-Barr virus [11, 14, 42, 45]. This virus has the ability to immortalize B-lymphocytes and it was demonstrated that the Epstein-Barr virus LMP1 antigen modulates the phenotype of tumor cells in Hodgkin's disease and induces in vitro morphologically normal cells to transform into Hodgkin/Reed-Sternberg-like cells

[10, 11, 21]. Furthermore, EBV-positive lymphocytes obtained from Hodgkin's disease were able to generate EBV-associated tumors in SCID mice [17, 32]. This suggests that EBV-antigens may be present in precursor cells and that these antigens might have the ability to transform precursor cells into tumor cells. However, it is believed that EBV associated antigens do not initiate the transformation process in Hodgkin's disease. Therefore, other sofar unidentified non-EBV antigens may be of importance for the initiation of tumor cell development.

Another important event during the differentiation process of B-lymphocytes is the rearrangement of immunoglobulin genes and the occurrence of somatic (hyper)mutations[4]. Furthermore, it is suggested that lymphocytes with mutated rearranged immunoglobulin genes may undergo other genomic alterations, such as deletions, duplications and translocations [8, 20]. For Hodgkin's disease, it is already demonstrated that Hodgkin/Reed-Sternberg cells contain immunoglobulin gene rearrangements with somatic hypermutations and have also acquired genomic instability [1, 5, 12, 16, 25, 26, 30, 34, 36, 37, 39, 40, 44]. The clonal derivation of primary Hodgkin/Reed-Sternberg cells from genetically aberrant but morphologically normal cells, i.e. the precursor cells, has to be demonstrated in future studies by detection of immunoglobulin gene rearrangements from single cells of both fractions[22, 23, 41]. The immunoglobulin rearrangement status of the precursor cell relative to the Hodgkin/Reed-Sternberg cell is of vital importance to determine whether the precursor cell has a naïve lymphocyte configuration or has acquired somatic mutations[43]. Some of the mutations acquired during the transformation into Hodgkin/Reed-Sternberg cell might be "crippling" mutations. Such mutations may block on the one hand the maturation potential and on the other hand block the apoptosis machinery, which would otherwise be activated.

Apoptosis is used for the antigen-driven selection of lymphocytes in the follicle centre. It is most evident in the light zone of the follicle centre, in which the centrocytes reside. It is suggested that immunoglobulin-negative centroblasts are unaffected by apoptosis. This indicates that (antigen specific) cell death in the follicle centre may be mediated by surface immunoglobulin engagement[19]. Therefore, cells which have surface immunoglobulins, but have lost antigen binding or show a reduced antigen binding, may die due to the activation of the apoptotic pathway [31]. Moreover, it is believed that the apoptotic events are triggered before the onset of somatic mutations [27]. All this, and the observation of (anti)apoptotic markers such as bax, fas, bcl-2 and bcl-x in Hodgkin/Reed-Sternberg cells , indicate that they may be derived from immunoglobulin-negative centroblasts or from centrocytes with an inadequate antigen binding capacity due to the failure of Hodgkin/Reed-Sternberg cells to activate the apoptotic machinery [2, 29, 33, 38, 46]. Consequently, the precursor cells also originate from centroblasts or centrocytes and, similar to the Hodgkin/Reed-Sternberg cells, fail to trigger apoptosis. However, the exact molecular mechanism of cell death inhibition in precursor cells and Hodgkin/Reed-Sternberg cells is still an enigma and has to be elucidated in future studies.

A striking difference between centroblasts and centrocytes is their proliferative capacity. Centroblasts are highly proliferative, whereas centrocytes do not divide [4, 18, 27]. Proliferation markers have been detected in Hodgkin/Reed-Sternberg cells. However, only a very small population of tumor cells (about 1%) is detected in the tumor area, implying a low frequency of cell division for Hodgkin/Reed-Sternberg cells. Therefore, the Hodgkin/Reed-Sternberg cells resemble more likely the centrocytes than centroblasts, not only due to their proliferative capacity but also to their mutations in the rearrranged immunoglobulin genes. The phenotype of precursor cells, on the other hand, most probably matches with a (pre)centroblast phenotype, but conclusive evidence has to be collected.

Finally, receptors and cytokines involved in the interactions between T-helper cells and

Hodgkin/Reed-Sternberg cells may be of importance in the transformation process and, therefore, have to be characterized for the interaction between T-helper cells and precursor cells. T-lymphocytes in the follicle centre and surrounding the Hodgkin/Reed-Sternberg cells in Hodgkin's disease are (activated) CD4-positive T cells [9, 18]. Cytokines, such as IL-1, IL-2, IL-6, TNF, CD30-ligand, CD40-ligand and CD95-ligand are important for the interaction between Hodgkin/Reed-Sternberg cells and T-lymphocytes, but may also important for the interaction between precursor cells and T-lymphocytes. On the other hand, IL-4 and IFN-g are cytokines which are involved in polykaryon formation [9]. One may speculate that due to the above-mentioned interactions, T-lymphocytes may induce polykaryon formation in precursor cells via IL-4 and IFN-g secretion, and therefore, transform them into Hodgkin/Reed-Sternberg cells. However, other cytokines and receptors might be of importance for the initiation of tumor cell development in Hodgkin's disease.
Future studies in Hodgkin's disease have to elucidate the molecular mechanisms involved in the transformation of precursor cells into Hodgkin/Reed-Sternberg cells.

Conclusion

This thesis shows that the malignant cell population in Hodgkin's disease is not limited to the Hodgkin/Reed-Sternberg cells by identifying a small precursor cell population that have numerical chromosome abnormalities. These precursor cells have the ability to proliferate and to differentiate into Hodgkin and Reed-Sternberg cells. During the process of differentiation genetic aberrations accumulate in the Hodgkin/Reed-Sternberg cells. Finally, on basis of the observed genetic relationships, the precursor cells are suggested to be progenitor cells of the Hodgkin/Reed-Sternberg cell population.

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