American Journal of Pathology, Vol. 145, No. 1, July 1994

Short Communication

Absence of HLA Class I Expression by Reed-Sternberg Cells

Sibrand Poppema and Lydia Visser

From the Cross Cancer Institute, Edmonton, Alberta, Canada

The reactive cell population in Hodgkin's disease consists of predominantly CD4$^+$ helper T cells and lacks CD8$^+$ cytotoxic T cells and natural killer cells. This lack of a CD8$^+$ response is surprising in view of the expression of the latent Epstein-Barr viral protein LMP by Reed-Sternberg cells in many cases of Hodgkin's disease. Deficient HLA class I expression would be one possible mechanism to avoid a CD8$^+$ cytotoxic immune response. To test this possibility we studied the expression of HLA class I and II determinants on Reed-Sternberg cells in tissue sections and cell suspensions of Hodgkin's disease. Frozen tissue sections of 40 cases and cytocentrifuge preparations from cell suspensions of 10 lymph nodes involved by Hodgkin's disease were studied with monoclonal antibodies reactive with HLA determinants. As a control frozen tissue sections of two cases of infectious mononucleosis were studied. Careful examination of the tissue sections and subsequently of cytospins of cell suspensions showed that the Reed-Sternberg cells frequently lacked HLA class I but showed strong staining for HLA class II. Absence of HLA class I expression on Reed-Sternberg cells and their variants provides an explanation for the lack of a CD8$^+$ cytotoxic immune response against antigens expressed on Reed-Sternberg cells. (Am J Pathol 1994, 145:37–41)

Hodgkin's disease is characterized by the presence of a small population of transformed cells, the Reed-Sternberg cells and variants (R-S cells), and a majority of reactive cells including lymphocytes, eosinophils, histiocytes, and plasma cells. The R-S cells are a clonal cell population based on the presence of clonal chromosomal abnormalities.[1]

Epstein-Barr virus (EBV)-associated DNA, RNA, or proteins can be found in R-S cells of up to 40% of cases of Hodgkin's disease.[2–5] In contrast to infectious mononucleosis where CD8$^+$, CD45$^+$ cytotoxic T cells predominate,[6] in the vast majority of cases of mixed cellularity and nodular sclerosis subtype of Hodgkin's disease, the involved tissues show a preponderance of CD4$^+$ T cells,[7–12] with an average CD4/CD8 ratio of 3.5.[11] Furthermore, CD8$^+$ T cells are conspicuously lacking among the lymphocytes immediately surrounding R-S cells.[12] Instead, there is a predominance of CD4$^+$, CD45R0$^+$, and CD45RB$^+$ interleukin-2-producing T cells, a phenotype consistent with Th1 cells as present in delayed-type hypersensitivity reactions.[13]

Absence of a cytotoxic T cell reaction may result from several mechanisms. A deficiency of CD8 cells is not likely because in the peripheral blood an increased proportion of CD8 cells are found.[14] Absence of antigen expression can also be excluded as a general mechanism because in EBV-positive Hodgkin cases LMP, which is one of the major targets for a cytotoxic immune response,[15] is strongly expressed on the membranes of the R-S cells.[5] A deficiency of the EBV-specific CD8 T cell population is unlikely because of the previous successful recovery of infectious mononucleosis in many of these patients.[16]

Finally, CD8 T cells recognize antigen only with the appropriate HLA class I antigen. Therefore, a defect involving HLA class I expression might compromise

Supported by a grant from the National Cancer Institute of Canada.

Accepted for publication March 21, 1994.

Address reprint requests to Dr. Sibrand Poppema, Department of Laboratory Medicine, Cross Cancer Institute, 11560 University Avenue, Edmonton, Alberta T6G 1Z2, Canada.

a cytotoxic immune response. Because LMP2 is generally expressed through HLA-A2.1[17,18] it has been hypothesized that EBV-positive Hodgkin's disease might be more prevalent among HLA-A2-negative individuals.[17] However, in a study of 72 cases of Hodgkin's disease we found no correlation between HLA-A2 expression and the presence or absence of EBV in R-S cells.[19]

Down-regulation of HLA is a mechanism used by several viruses and tumors to escape the immune response.[20] In this study we analyze the expression of monomorphic determinants of HLA class I, β_2-microglobulin, the polymorphic HLA-A2 epitope, a monomorphic determinant of HLA class II, and HLA class II invariant chain in R-S cells and their mononuclear variants.

Materials and Methods

A total of 40 cases of Hodgkin's disease including all major subtypes was studied on frozen tissue sections. In addition, two cases of infectious mononucleosis were studied. Because R-S cells are generally surrounded by other cell types and only cells that are adjacent to another R-S cell can be evaluated in tissue sections, we also studied 10 cases on cytospin preparations of cell suspensions prepared from involved lymph nodes. The antibody against a polymorphic HLA-A2 epitope (clone BB7.2) was obtained from Incstar (Stillwater, MN) and antibodies reactive with monomorphic determinants of HLA class I (HLA A, B, C) were obtained from Incstar (clone MB40.5), Serotec, Oxford, UK (clone W6/32), and Immunotech, Marseille, France (clone B9.12.1). The anti-β2-microglobulin antibody was obtained from Immunotech (clone B1G6) and antibodies against HLA class II (MB4) and invariant chain (MB3) were prepared in our own laboratory. An indirect immunoperoxidase method using a second step peroxidase-conjugated goat anti-mouse Ig antibody (Jackson Laboratories, Bar Harbor, ME) and 3-amino-9-ethylcarbazole as a peroxidase substrate were used.

Results

The immunohistochemical analysis of HLA expression on frozen tissue sections in the 40 cases showed positive staining for monomorphic HLA class I determinants in all cases and positive staining for the polymorphic HLA-A2 determinant in approximately 50% of the cases. Positive cases were identified by the staining of membranes of lymphocytes and other cell types in the frozen tissue sections. The antibodies reactive with HLA class II stained R-S cells in all cases.

Analysis of R-S cell staining by the polymorphic HLA-A2 and the monomorphic HLA-ABC determinants on frozen tissue sections was frequently difficult because most R-S cells are surrounded by a rim of HLA class I-positive lymphocytes. However, in 22 cases clusters of adjoining R-S cells could be identified that showed negative or very weak staining of R-S cells (Figure 1A). These cells were strongly positive for HLA class II (Figure 1B). Also, groups of adjoining histiocytes showed strong staining for HLA class I and II. In 12 cases the staining could not be evaluated because all R-S cells were immediately surrounded by HLA class I-positive reactive cells. In six cases, including all four lymphocyte predominance cases studied, the R-S cells stained positive for the monomorphic determinants of HLA class I (Table 1).

Analysis on cytospin preparations of cell suspensions of 10 cases confirmed the absence of HLA class I on the surface and in the cytoplasm of the R-S cells (Figure 2A). The same results were obtained with three different antibodies reactive with anti-monomorphic HLA class I determinants and with the anti-β_2-microglobulin antibody. In four cases we also used a more sensitive detection system using a biotinylated

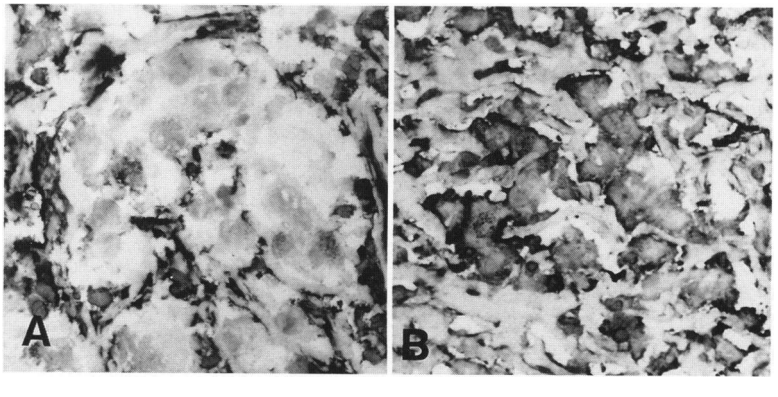

Figure 1. *Immunoperoxidase staining of cluster of lacunar-type R-S cells in frozen tissue sections of a case of nodular sclerosis subtype of Hodgkin's disease. The R-S cells do not stain for HLA class I, whereas lymphocytes and fibroblasts show strong staining* (**A**). *Staining for HLA class II shows a reversed pattern with strong staining of R-S cells and absence of staining on lymphocytes* (**B**).

Table 1. *HLA Class I Expression in R-S Cells*

	Tissue			Suspension		
	Pos	Neg	NI*	Pos	Neg	NI
Lymphocyte predominance	4	0	0	1	0	0
Nodular sclerosis	1	17	10	0	7	0
Mixed cellularity	1	5	2	1	1	0

* NI, not interpretable

second step and streptavidin alkaline phosphatase as a third step and obtained identical results. The anti-HLA class II and anti-invariant chain antibodies showed strong staining of R-S cells (Figure 2B). In all cases staining for lymphocyte subsets showed a predominance of $CD4^+$ cells. In the lymphocyte predominance cases many CD4 cells also expressed CD57.

The two cases of infectious mononucleosis showed positive staining for HLA class I and II and staining for lymphocyte subsets showed a predominance of $CD8^+$ cells.

Discussion

Absence of HLA class I on R-S cells was found to be common in nodular sclerosis and mixed cellularity but not lymphocyte predominance subtypes of Hodgkin's disease. On the other hand, HLA class II was consistently and strongly present on R-S cells. In normal tissues, HLA class I is expressed on nearly all cells except trophoblast of the placenta. Loss of HLA class I monomorphic determinants has been reported in viral infections and melanomas[21] and several other tumors[22] as well as in mouse tumor models.[23] This loss is thought to be associated with increased malignancy.[24] Selective loss of a polymorphic HLA-A2 determinant has been found in several tumors.[25] Selective loss of HLA-A11 has been shown in Burkitt's lymphoma.[26]

The mechanisms leading to partial or complete loss of HLA may be variable. The expression of HLA class I is tightly regulated and altered binding of at least two different regulatory factors (KBF1 and NF-kB) to the HLA class I enhancer sequence may result in diminished expression.[27] It has been shown that adenovirus can induce loss of HLA class I expression in a rat tumor cell line model,[23] resulting in evasion of T cell immunity.[24] The mechanism involves an major histocompatibility complex binding protein that blocks transport of class I to the cell surface.[28]

Loss of HLA determinants has been found in recirculating lymphocytes in nonsymptomatic individuals with HTLV1 infection.[29] Viral infection may directly switch off HLA expression or lead to selection of cells with diminished HLA expression. As a result, the virus-infected cell may escape the immune response. Similarly, tumors may escape the immune response by down-regulation of HLA class I determinants. Although cytotoxic T cells require recognition of antigen with HLA class I, natural killer cells are possibly activated by the absence of HLA class I antigens.[30] In cytomegalovirus infection, a virus-induced protein, H301, is produced that binds to β_2-microglobulin and blocks transport of HLA class I antigen to the cell surface. This results in reduced lysis by cytotoxic T cells but is also a signal for increased natural killer cell recognition and lysis.[16] However, tissues involved by Hodgkin's disease contain very few cells with a phenotype consistent with natural killer cells.[12] The mechanisms determining the balance between HLA expression and specific cytotoxic versus natural killer cell recognition and lysis are not fully understood.

In a previous study[31] we found that Hodgkin cell lines L428[32] and ZO[33], which have classical R-S cell immunophenotypes (ie, no T or B cell markers and CD15 and CD30 positive), do not express HLA class I, whereas EBV-transformed lymphoblastoid B cell lines are positive. Cases of Hodgkin's disease that are EBV positive follow infectious mononucleosis by approximately 2 years.[16] The immunoblasts and multinucleated cells in the two cases of infectious mononucleosis expressed HLA class I and II and were accompanied by a prominent $CD8^+$ T cell population. This clearly contrasts with the predominant $CD4^+$ lymphocyte infiltrate in Hodgkin's disease.[7–12]

It is of interest that the infiltrating lymphocytes in Hodgkin's disease lesions have a phenotype consistent with Th1 cells and on stimulation *in vitro* can be shown to produce interleukin-2.[13] We have not been able to demonstrate production of interleukin-2 and interferon-γ by these cells *in situ*. Interferon-γ can increase the expression of HLA antigens on some cell types. It is possible that R-S cells produce factors, such as the EBV gene product BCRF1 and its host homologue interleukin-10, that can inhibit the production of interferon-γ by the Th1 lymphocytes.[34]

Based on these findings, the following scenario for the development of Hodgkin's disease in EBV-positive patients can be proposed. EBV transforms B cells that initially have normal expression of EBV-induced latent antigens (such as EBNA 1, 2, 3A, 3B, 3C, and LMP1 and LMP2) and normal HLA class I expression. This provides the basis for an efficient specific cytotoxic immune response against a variable number of these antigens, dependent on their HLA type. In some individuals, selection of a cell with diminished expression of latent proteins and HLA class I leads to an escape from the CD8-mediated

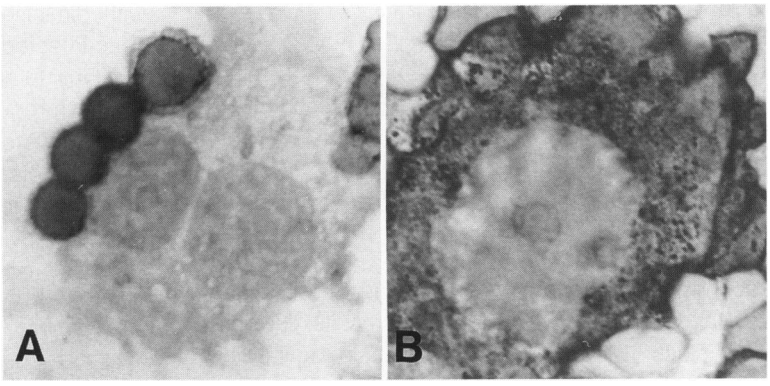

Figure 2. *Immunoperoxidase staining of lacunar-type of R-S cells in cytocentrifuge preparations of a case of nodular sclerosis subtype of Hodgkin's disease. The R-S cells were entirely negative for HLA class I, whereas the lymphocytes were strongly positive* (**A**). *Staining for HLA class II shows a reversed pattern with strong staining of the R-S cells and positive staining of a proportion of the lymphocytes* (**B**).

cytotoxic immune response. However, the simultaneously maintained expression of antigen in association with HLA class II provides the set up for a CD4-mediated immune reaction. The result is a tumor with a relatively small clone of HLA class I-negative, HLA class II-positive neoplastic cells that induce a delayed-type hypersensitivity reaction.

EBV-negative cases also lack HLA class I, strongly express HLA class II, and show a CD4-mediated delayed-type hypersensitivity reaction. Therefore, the mechanism is likely to be similar, but a pathogenic agent has not been identified in these cases.

The implication of our findings is that induction of HLA class I with interferon-γ or other approaches may theoretically contribute to re-establishment of an effective cytotoxic immune response against the R-S cells.

References

1. Poppema S, Kaleta J, Hepperle B: Chromosomal abnormalities in patients with Hodgkin's disease: evidence for frequent involvement of the 14q chromosomal region but infrequent bcl-2 gene rearrangement in Reed-Sternberg cells. J Natl Cancer Inst 1992, 84: 1789–1793
2. Poppema S, van Imhoff G, Torensma R, Smit J: Lymphadenopathy morphologically consistent with Hodgkin's disease associated with Epstein-Barr virus infection. Am J Clin Pathol 1985, 84:385–390
3. Weiss LM, Strickler JG, Warnke RA, Purtilo DT, Sklar J: Epstein-Barr viral DNA in tissues of Hodgkin's disease. Am J Pathol 1987, 129:86–91
4. Staal SP, Ambinder R, Beschorner WE, Hayward GS, Mann R: A survey of Epstein-Barr virus in lymphoid tissue: frequent detection in Hodgkin's disease. Am J Clin Pathol 1989, 91:1–5
5. Young LS, Deacon EM, Rowe M, Crocker J, Herbst H, Niedobitek G, Hamilton-Dutoit SJ, Pallesen G: Epstein-Barr virus latent genes in tumour cells of Hodgkin's disease. Lancet 1991, 337:1617
6. Abbondanzo SL, Sato N, Straus SE, Jaffe ES. Acute infectious mononucleosis. CD30 (Ki-1) antigen expression and histologic correlations. Am J Clin Pathol 1990, 93:698–702
7. Poppema S, Bhan AK, Reinherz EL, Posner MR, Schlossman SF: In situ immunological characterization of cellular constituents in lymph nodes and spleens involved by Hodgkin's disease. Blood 1982, 59:226–232
8. Aisenberg AC, Wilkes BM: Lymph node T cells in Hodgkin's disease: Analysis of suspensions with monoclonal antibody and rosetting techniques. Blood 1982, 59:522
9. Dorreen MS, Habeshaw JA, Wrigley PFM, Lister TA: Distribution of T lymphocyte subsets in Hodgkin's disease characterized by monoclonal antibodies. Br J Cancer 1982, 45:491–499
10. Borowitz MJ, Croker BP, Metzgar RS: Immunohistochemical analysis of the distribution of lymphocyte populations in Hodgkin's disease. Cancer Treat Rep 1982, 66:667–674
11. Martin JM, Warnke RA: A quantitative comparison of T-cell subsets in Hodgkin's disease and reactive hyperplasia: frozen section immunohistochemistry. Cancer 1984, 53:2450–2455
12. Poppema S: The nature of the lymphocytes surrounding Reed-Sternberg cells in nodular lymphocyte predominance and in other types of Hodgkin's disease. Am J Pathol 1989, 135:351–357
13. Poppema S, Kaleta J: Biology of Hodgkin's disease, a model based on viral infection and delayed type hypersensitivity reaction. Ann Oncol 1992, 3:5–8
14. Romagnani S, Del Prete GF, Maggi E: Displacement of T lymphocytes with the "helper/inducer" phenotype from peripheral blood to lymphoid organs in untreated patients with Hodgkin's disease. Scand J Haematol 1983, 31:305–314
15. Murray RJ, Wang D, Young L, Wang F, Rowe M, Kieff E, Rickinson AB: Epstein-Barr virus-specific cytotoxic T-cell recognition of transfectants expressing the virus-coded latent membrane protein LMP. J Virol 1988, 62:3747–3757
16. Mueller N, Evans A, Harris NL, Comstock GW, Jellum E, Magnus K, Orentreich N, Polk BF, Vogelman J:

Hodgkin's disease and Epstein-Barr virus. Altered antibody pattern before diagnosis. N Engl J Med 1989, 320:689–695
17. Murray RJ, Kurilla MG, Brooks JM, Thomas WA, Rowe M, Kieff E, Rickinson AB: Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies. J Exp Med 1992, 176:157–168
18. Khanna R, Burrows SR, Kurilla MG, Jacob CA, Misko IS, Sculley TB, Kieff E, Moss DJ: Localization of Epstein-Barr virus cytotoxic T cell epitopes using recombinant vaccinia: implications for vaccine development. J Exp Med 1992, 176:169–176
19. Poppema S, Visser L: Epstein-Barr virus positivity in Hodgkin's disease does not correlate with HLA A2 negative phenotype. Cancer 1994 (in press)
20. Maudsley DJ, Pound JD: Modulation of MHC antigen expression by viruses and oncogenes. Immunol Today 1991, 12:429–431
21. Ruiter DJ, Bhan AK, Harrist TJ, Sober AJ, Mihm MC: Major histocompatibility antigens and mononuclear inflammatory infiltrate in benign nevomelanocytic proliferations and malignant melanoma. J Immunol 1982, 129:2808–2815
22. Elliott BE, Carlow DA, Rodricks A-M, Wade A: Perspectives on the role of MHC antigens in normal and malignant cell development. Adv Cancer Res 1989, 53:181–245
23. Schrier PI, Bernhards R, Vaessen RTMJ, Houweling A, van der Eb AJ: Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. Nature 1983, 305:771–775
24. Bernhards R, Schrier PI, Houweling A, Bos JL, van der Eb AJ: Tumorigenicity of cells transformed by adenovirus type 12 by evasion of T-cell immunity. Nature 1983, 305:776–779
25. Natali PG, Nicotra MR, Bigotti A, Venturo I, Marcenaro L, Giacomini P, Russo C: Selective changes in expression of HLA class I polymorphic determinants in human solid tumors. Proc Natl Acad Sci USA 1989, 86: 6719–6723
26. Masucci MG, Torsteinsdottir S, Colombani J, Brautbar C, Klein E, Klein G: Down-regulation of class I HLA antigens and of the Epstein-Barr virus-encoded latent membrane protein in Burkitt lymphoma lines. Proc Natl Acad Sci USA 1987, 84:4567–4571
27. Blanchet O, Bourge J-F, Zinsner H, Israel A, Kourilsky P, Dausset J, Degos L, Paul P: Altered binding of regulatory factors to HLA class I enhancer sequence in human tumor cell lines lacking class I antigen expression. Proc Natl Acad Sci USA 1992, 89:3488–3492
28. Andersson M, Paabo S, Nilsson T, Peterson PA: Impaired intracellular transport of class I MHC antigens as a possible means for adenoviruses to evade immune surveillance. Cell 1985, 43:215–222
29. Sonoda S, Yashiki S, Takahashi K, Arima N, Daitoku Y, Matsumoto M, Matsumoto T, Tara M, Shiamyozu K, Sato K, Inoko H, Ando A, Tsuji K: Altered HLA antigens expressed on T and B lymphocytes of adult T-cell leukemia/lymphoma patients and their relatives. Int J Cancer 1987, 40:629–634
30. Karre K, Ljunggren HG, Piontek G, Kiessling R: Selective rejection of H-2 deficient lymphoma variants suggests an alternative immune defence strategy. Nature 1986, 319:675–678
31. Poppema S, Brinker MGL, Visser L: Evidence for a B cell origin of the proliferating cells. In: Hodgkin's disease in children: controversies and current practice. Kluwer Academic publishers, Boston, 1988, pp. 5–27
32. Schaadt M, Diehl V, Stein H, Fonatsch C, Kirchner HH: Two neoplastic cell lines with unique features derived from Hodgkin's disease. Int J Cancer 1980, 26:723–731
33. Poppema S, Visser L, de Jong B, Brinker M, Atmosoerodjo J, Timens W: The typical Reed-Sternberg phenotype and Ig gene rearrangement of Hodgkin's disease derived cell line ZO indicating a B cell origin. Rec Res Cancer Res 1989, 117:67–74
34. Mosmann TR: Role of a new cytokine, interleukin 10, in the cross-regulation of T helper cells. Ann NY Acad Sci 1991, 628:337–344