Immune escape mechanisms in Hodgkin’s disease

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Summary

Background: The nodular sclerosis and mixed cellularity subtypes of Hodgkin’s disease are histologically characterised by a small population of neoplastic cells, the so-called Reed-Sternberg cells and their mononuclear variants (RS cells) and an extensive admixture of other cell types including lymphocytes, plasma cells, eosinophils, and histiocytes. The nature of this infiltrate is largely known, but the mechanisms and functional effects are not. The small lymphocytes immediately surrounding the RS cells are mostly CD4+ T cells that express early activation markers. The absence of prominent specific cytotoxic T cell or natural killer (NK) cell populations seems to argue against a Th1-type response, whereas the sometimes prominent admixture of plasma cells and eosinophils is suggestive of a Th2-type response. Enrichment of the CD4 T-cell population may result from selective influx of CD4 T cells or from selective depletion of CD8 cells and NK cells.

Results and discussion: The T cells surrounding RS cells have an immuno-phenotype and cytokine production capability consistent with a Th2-type response. RS cells express several members of the TNF receptor family such as the FAS ligand (CD95L) that may induce apoptosis of activated, FAS expressing, CD8+ T cells and NK cells. The RS cells also produce TGFB and interleukin-10 that may downmodulate the Th1 response. In addition, the Reed-Sternberg cells produce the chemokine TARC that could lead to the specific attraction of a Th2 T-cell subset.

Conclusion: RS cells have several mechanisms that may allow it to escape an effective immune response. The relative contributions of each of these and other potential mechanisms are not yet known.

Key words: Hodgkin’s disease, Th2, chemokine, TARC, FAS, FASL, TGFβ, IL-10

Characterisation of the cells surrounding the RS cells

The lymphocytes in close vicinity of HRS cells are almost exclusively positive for CD4, while essentially no CD8+ or NK-cells are present in this area [1]. These T cells are not clonal and are not part of the neoplastic population of RS cells which have no B- or T-cell markers but frequently do have immunoglobulin gene rearrangements [2]. One possible reason for the presence of an extensive inflammatory infiltrate would be an immune response against the neoplastic cells. In light of the presence of the EB virus [3, 4] and at least some EBV-induced membrane antigens (LMP1, 2) in the RS cells of many cases of Hodgkin’s disease, this would be a possibility [5].

The increase of CD4+ cells in Hodgkin nodes is associated with a decrease in CD4+ cells in the circulation and predominantly reflects increased influx of mature CD4+ T lymphocytes into the involved tissues [6]. Results of imaging with indium-labelled lymphocytes showing positivity in involved nodes support this concept [7]. Activated CD8+ T cells, as determined by granzyme positivity, have been described in some cases but not immediately surrounding the HRS cells. Surprisingly, the presence of these granzyme positive lymphocytes was associated with an unfavourable prognosis [8].

The CD4+ T cells that surround the RS cells are in a state of activation as indicated by the presence of several activation associated surface markers, including CD38, CD69, CD71, and HLA class II [9 10]. A considerable number of these lymphocytes have TIA-1 positive granules, but the significance of this finding is not clear. There are no indications that the lymphocytes are of clonal origin or have a restricted TCR beta gene variable region repertoire [11, 12]. The lymphocytes lack expression of another activation marker, CD26 (dipeptidylpeptidase IV) [13], a surface molecule involved in co-stimulation of T lymphocytes [14]. Further characterisation of CD4+ T cells derived by flow cytometry from lymph nodes of classical as well as NLP Hodgkin’s disease, revealed that they are predominantly CD45RO+/CD45RBdim, suggesting an activated/memory Th2 phenotype [15]. When the total lymph node cell population is optimally stimulated in vitro with phorbolester and ionomycin IL-2, IFNγ as well as IL-4 are produced. When the CD4+/CD26− lymphocytes that immediately surround the HRS cells are sorted from Hodgkin lymph nodes, these cells do not produce IL-2 but do produce IFNγ and increased amounts of IL-4 when optimally stimulated [15, 16].

In vitro, naïve CD4+ T cells can be primed for a Th2 response by anti-CD28 without anti-CD3 in the pres-
ence of IL-2. Approximately 30% of T cells primed in this way express CD57 [17]. CD57+ Th2-primed clones produced three times as much IL-4 and IL-5 than the CD57- Th2-primed clones and also produced IFNγ. Interestingly, the T cells in the nodular lymphocyte predominance subtype have a CD57+/CD4+ phenotype [10, 18]. Improper stimulation of the T cells by co-stimulatory molecules such as B7.1 and B7.2 on the RS cells [19] could lead to this unusual Th2-like cytokine production. This may explain the observed Th2-immunophenotype of the T cells in Hodgkin’s disease (CD45RBdim/CD26–), but also the absence of a cytotoxic response against RS cells.

Anergy of surrounding T cells as a possible cause for impaired cytotoxicity

As mentioned earlier, the T cells surrounding the RS cells in the classical types of Hodgkin’s disease do not express CD26, although more than 60% of normal peripheral blood and lymph node T cells are CD26+ [9]. CD26 physically interacts with adenosine deaminase (ADA) and with CD45R0, both of which are important in immune response [13, 14]. Normal CD26– T cells become CD26+ by stimulation with antigens/mitogens under physiological conditions [20, 21], but the CD26– cells from Hodgkin lesions remain negative after stimulation [9]. This indicates that the absence of CD26 is potentially relevant with respect to the impaired immune response observed in Hodgkin’s disease. Selected CD26– lymphocytes from cases of classical Hodgkin’s disease can be stimulated in vitro to produce IFNγ and IL-4, but not IL-2 [9]. The inability to produce IL-2 and reduced proliferation are the hallmarks of anergic T cells.

Anergic T cells can be obtained by several mechanisms: lack of co-stimulation [22, 23], activation by superantigens [24], or the effect of certain cytokines (IL-10, TGFβ) [25, 26]. Thus, a possible way for HRS cells to escape cytotoxic killing is by induction of anergy in T cells. The anergic state of the lymphocytes is probably not the result of a lack of co-stimulation by CD80 (B7.1), CD86 (B7.2), and various other adhesion/co-stimulatory molecules such as CD58 (LFA-3) and CD54 (ICAM-1), because these molecules are highly expressed on HRS cells [19, 27].

HRS cells are capable of producing a wide variety of cytokines including IL-10 and TGFβ [27–29]. IL-10 and TGFβ are known to be capable of anergy induction [30–32]. Supernatant from Hodgkin cell line L428 inhibits CD25 expression and IL-2 production when peripheral blood mononuclear cells are stimulated with anti-CD3, while the cells still become CD69 positive. This indicates that a soluble factor is responsible for the improper activation [32]. L428 is known to produce TGFβ and IL-10 [33], and depletion of TGFβ from L428 supernatant completely prevents the inhibitory effect [32]. Adding the removed TGFβ to RPMI gave a similar reduced CD25 and IL-2 expression pattern as with L428 supernatant [32]. IL-10 depletion did not affect the inhibitory effect of L428 supernatant. These findings indicate that TGFβ is the T-cell inhibitory factor in the L428 cell line.

TGFβ is generally produced in a latent, inactive complex composed of the bio-active TGFβ homodimer (25 kD) and a non-covalently bonded precursor protein [34]. Often a latent TGFβ binding protein is associated with this complex. The bio-active TGFβ homo-dimer can be released from the complex after very strong acidification. The TGFβ produced by the L428 cell line is already active at physiological pH and has a much higher molecular weight than the usual active form [36]. By using SDS-PAGE the L428 TGFβ was shown to contain a 25 kD molecule that crossreacted with antibodies against TGFβ. High molecular weight TGFβ was also observed in the urine of patients with Hodgkin’s disease while it was absent from healthy controls, indicating that it is also produced in vivo by HRS cells [35]. Production of TGFβ that is already active at physiological pH by HRS cells may enable them to (de)regulate the immune response in their favour. TGFβ is also a potent growth factor for fibroblasts and is known to promote the formation of extracellular matrix and fibrosis [34]. The L428 cell line was derived from a patient with nodular sclerosis subtype of Hodgkin’s disease. Therefore, TGFβ may shape the environment for HRS cells by suppressing the T-cells response and inducing the collagen formation in the nodular sclerosis subtype.

Possible role for members of the TNFR/NGFR and TNFL superfamilies

CD30, now identified as member of the TNFR family, was first discovered with a monoclonal antibody prepared against the cell line L428 that showed a consistently high expression on HRS cells [36]. Further investigations revealed that CD30 expression is not restricted to HRS cells alone, but is also expressed on activated T and B lymphocytes. The abundant presence of CD30 on HRS cells and the absence in most non-Hodgkin lymphomas suggests that it may play an important role in the development of Hodgkin’s disease. The TNFR superfamily has gradually grown during recent years [37]. Their natural ligands form two superfamilies, the neutrophins (NGFL superfamily), and the TNFL superfamily. Several members of both the TNFR and TNFL superfamily have been observed in Hodgkin’s disease. CD27L (CD70), CD40, and CD95 (Fas/Apo) are highly expressed on HRS cells, but the expression of CD120a and CD120b is also seen [16]. Some of their ligands, notably CD30L and CD40L, are expressed on the surrounding activated T cells [38, 39]. These T cells also express CD95 but only minimal amounts of CD95L. Somewhat surprisingly, CD40L is absent from the CD57+ lymphocytes in NLP-Hodgkin’s disease (Poppema, unpublished). Several members of these superfami-
lies have an important role in the regulation of proliferation and apoptosis [40, 41]. CD40/CD40L interaction is extremely important in B-cell activation. CD95/CD95L interaction is capable of apoptosis induction.

CD95/CD95L-induced apoptosis is involved in the maintenance of immune privilege and peripheral tolerance [41]. It is also one of the mechanisms used in the cytotoxic response by T cells. It is therefore interesting that there is no adequate cytotoxic response towards HRS cells, although they express substantial amounts of CD95.

Possible mechanisms for HRS cells to escape a cytotoxic response are by deletion of reactive cytotoxic T cells and NK cells, or by deletion of Th1 cells, resulting in the absence of help to the effector cells. Th1 cells are more sensitive than Th2 cells to CD95 mediated activation induced cell death [42]. This difference in susceptibility is already present in TH0 cells [43]. In Hodgkin’s disease, the surrounding lymphocytes predominantly express a Th2 phenotype. A polyclonal antibody reactive with membrane bound as well as secreted CD95L and a monoclonal antibody reactive with a cytoplasmic determinant of CD95L give strong staining of HRS cells. CD95L expression has also been detected on malignant cells in several other malignancies, giving these malignant cells yet another possible mechanism to escape the immune response [44]. It is therefore possible that HRS cells escape a cytotoxic and Th1 immune response through the induction of apoptosis in surrounding cytotoxic T and NK lymphocytes and Th1 cells.

Conclusion

Hodgkin’s disease is characterised by HRS cells surrounded by CD4+ T lymphocytes. These lymphocytes express a variety of activation markers, but are incapable of mounting an effective immune response against the HRS cells. The lymphocytes typically are CD45RO+/CD45RBdim, lack the expression of CD26, and in vitro can be stimulated to produce IFNγ and IL-4, but not IL-2, but do not spontaneously produce cytokines. The CD45 isotype expression, the absence of CD26, and the potential for IL-4 production suggest that these lymphocytes have a Th2 phenotype. Activation of T cells via the CD28 co-receptor in a cytokine-rich environment without appropriate stimulation via the TCR may lead to the IL-4 and IFNγ producing cytokine profile found in the T cells in Hodgkin tissues. Absence of IL-2 production is not only a characteristic of Th2 cells but also of anergic cells, indicating that the lymphocytes could also be in a state of anergy. Lack of IL-2 production, the induction of a predominant Th2 response, or the induction of anergy, would all contribute to an ineffective immune response against the HRS cells. Another potential factor responsible for the lack of an effective immune response is the cytokine TGFβ that is produced in an active form by HRS cells and has potent immunosuppressive effects on T cells as well as a fibrosis promoting effect. IL-10 that is also produced by some HRS cells also may modulate the immune response towards a Th2 type.

The TNF/TNFR superfamilies probably play an important role in Hodgkin’s disease, as several members of these families, notably CD30, CD40, CD70, CD95 and also CD95L are highly expressed on the HRS cells. Moreover, their natural ligands are expressed on the surrounding lymphocytes. A general feature of members of the TNF/TNFR family is their involvement in cell activation and/or apoptosis, and therefore they may play a crucial role in improper activation of the lymphocytes. Unequal susceptibility of lymphocyte subpopulations to Fas-mediated cell death could result in the absence of appropriate effector cells such as CD8+ T cells and NK cells, or appropriate helper cells like Th1 CD4+ cells.

Finally, a novel explanation for the specific phenotype of Hodgkin’s disease is offered by our recent finding that the tumor cells of the classical subtypes of Hodgkin’s disease produce high quantities of the CC chemokine TARC (reported at the fourth International Conference on Hodgkin’s Disease, Cologne, April 1998). This chemokine is normally produced in much smaller amounts by a subset of antigen-presenting cells and strongly attracts T lymphocytes expressing the CCR4 receptor. Activated Th2 lymphocytes indeed express the CCR4 receptor, whereas Th1 lymphocytes do not and express the CCR5 receptor. The production of TARC by the RS cells may therefore result in the specific attraction of Th2-type T lymphocytes.

In conclusion, there is a range of factors present in Hodgkin’s disease that may contribute to the paradox of an extensive inflammatory infiltrate and concomitant ineffectiveness of the host anti-tumor response. The increased knowledge of these factors may contribute to the design of biologicals that can interrupt the cycle of tumor cell proliferation and lymphocyte influx of Hodgkin’s disease in an effective and non-toxic manner.

References

6. Romagnani S. Del Prete OF, Maggi E et al. Displacement of T lymphocytes with the ‘helper/inducer’ phenotype from peripheral


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