Immune escape

Immune escape mechanisms in ALCL

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Why do host T cells not recognise and eradicate anaplastic large cell lymphomas?

Systemic anaplastic large cell lymphoma (ALCL) is a CD30 positive T cell lymphoma with a broad spectrum of morphological, immune phenotypical, and clinical characteristics.[1] Two clinicopathological entities can be distinguished: anaplastic lymphoma kinase (ALK) positive systemic nodal ALCL and ALK negative systemic nodal ALCL. ALK expression, usually the result of a t(2;5) translocation, is related to a younger age, lower international prognostic index risk, and an excellent prognosis.[2–4] Similar to most other lymphomas, ALCLs harbour many non-neoplastic, in principle immune competent, lymphocytes. In immune competent patients, putative expression of tumour antigens in ALCL (or any other lymphoma) should, in principle, elicit an antitumour immune response. Indeed, it was shown recently that ALK can elicit a humoral antitumour immune response in ALK positive patients with ALCL and that functional anti-ALK CTL precursors are present within the peripheral T cell repertoire of healthy donors, clearly indicating that ALK is a tumour antigen.[5 6] In addition, the epithelial tumour antigen MUC1 (also known as EMA) is highly expressed in ALK positive ALCL,[7] and MUC1 has been shown to elicit a MUC1 specific cytotoxic immune response in haematological malignancies.[8] However, the very presence of tumour cells indicates that any antitumour immune response, whether humoral or cytotoxic, is apparently insufficient for the elimination of tumour cells. Assuming the presence of a specific antitumour immune response, this indicates that tumour cells have acquired mechanisms to escape from this immune response.

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DO ALCLS HARBOUR TUMOUR CELL SPECIFIC INFILTRATING LYMPHOCYTES?

We have shown previously that activated CTLs (that is, granzyme B and CD3/CD8 positive lymphocytes) can be detected in all ALCLs, and that the numbers of activated CTLs vary considerably between individual cases.[2] T cells become activated only after recognition of specific antigens, and thus the presence of activated CTLs suggests the presence of a specific immune response against non-self antigens. However, there is no definite proof that these activated CTLs are actually directed against the tumour cells. Importantly, it appears that patients with ALCL who have many activated CTLs show a relatively poor response to chemotherapy and a poor clinical outcome.[2] We have explained this phenomenon by suggesting that the presence of a strong immune response will result in either efficient killing of the tumour cells, in which case no clinically detectable tumour will occur, or in the selection of tumour cells that have become resistant to the cell death inducing effect of CTLs. In this last case, if the inhibition of CTL induced tumour cell death is caused by interference with the downstream apoptosis cascade, this will also result in resistance to chemotherapy induced cell death,[9] and thus explain the poor clinical outcome in patients with many activated CTLs. However, apart from inhibition of (CTL induced) apoptosis, tumour cells have many pathogenic mechanisms by which they can escape from a CTL mediated cell death. In this short survey, we will discuss the possible mechanisms by which the neoplastic cells of ALCL may escape from a cytotoxic T cell mediated immune response. However, in contrast to Hodgkin's lymphoma, very few studies have investigated the presence of immune escape mechanisms in ALCL.

INHIBITION OF T CELL FUNCTION

Interference with antigen presentation

An effective cytotoxic immune response depends on an intact interaction between the T cell receptors on CTLs and the major histocompatibility complex (MHC) class I molecules associated with "non-self" peptides on the target cell, together with the appropriate costimulatory proteins. Downregulation of MHC class I molecules, which could protect the neoplastic cells against CTL recognition and killing, has been described in various tumours, including Burkitt's lymphomas and Hodgkin's disease.[10–13] However, the expression of MHC class I and II proteins and costimulatory proteins has not yet been studied in ALCL. Thus, the importance of downregulation of these molecules as an immune escape mechanism remains to be determined.

Inducing T cell anergy by secretion of immune modulatory cytokines

A complex array of cytokines and chemokines with very different, partly opposing, functions tightly controls the immune response against target cells. Expression of immune suppressive cytokines by the tumour cells may shift the balance towards tumour tolerance instead of tumour cell killing. Tumour cells have been shown to secrete immunosuppressive cytokines, possibly leading to both generalised inhibition of immune responses and local anergy or tolerance in tumour specific CTLs.[14] Again, few studies have been performed in ALCL, but one study clearly demonstrated the expression of interleukin 10 (IL-10) in ALCL.[15] IL-10 inhibits macrophages, causes downregulation of MHC class II molecules and cytokine synthesis by T helper type 1 cells, and has been shown to have a local effect on tumour cells, rendering them insensitive to CTL mediated lysis.[16]

Expression of FASL on neoplastic cells

Recently, an additional strategy that provides tumour cells with an advantage was described. Neoplastic cells of various non-lymphoid malignancies were found to express the FAS ligand (FASL), and to induce apoptosis of FAS expressing T cells infiltrating the tumours, both in vitro and in situ.[17] Thus, this offensive strategy might provide an alternative way to escape from a CTL mediated immune response.[18] In T cell lymphomas (including ALCL), varying degrees of FASL expression were found,[19 20] so that FASL expression might be involved as an immune escape mechanism in ALCL.

DISRUPTION OF THE INTRACELLULAR CTL INDUCED CELL DEATH SIGNALLING PATHWAY

CTL induced killing of target cells

After the recognition of target cells, activated CTLs induce cell death by the induction of apoptosis. Apoptosis is an ATP dependent physiological process with characteristic morphological features.[21] Upon induction of apoptosis, a cascade of proteases called caspases (cystein containing aspartic acid specific proteases) is activated. Once activated, these caspases dismantle the cell by selectively cleaving key proteins. In vitro studies have elucidated two major apoptosis pathways: a caspase 9 mediated

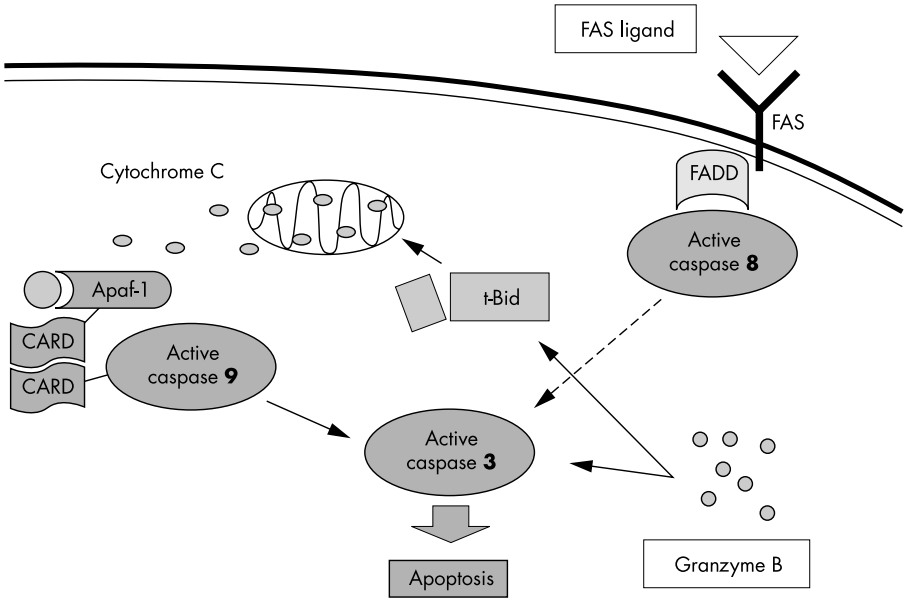

Figure 1 Schematic representation of cytotoxic T lymphocyte (CTL) induced apoptosis signalling pathways. When released into the cytoplasm of the target cell, granzyme B can activate caspase 3, both directly and indirectly, by truncation of the proapoptotic bcl-2 family member Bid (t-Bid), resulting in activation of the caspase 9 mediated pathway. Ligation of the FAS death receptor by CTL secreted FAS ligand will result in activation of caspase 8, again resulting in activation of caspase 3 with execution of apoptosis.

pathway, activated by DNA damage, and a caspase 8 mediated pathway, activated by ligation of specific death receptors, including Fas. Both pathways induce apoptosis via activation of effector caspases, in particular caspase 3.[22 23] CTLs can activate these apoptosis pathways in different ways (fig 1); namely: (1) via direct activation of caspase 3 by granzyme B; (2) via truncation of Bid by granzyme B, leading to cytochrome C release and caspase 9 mediated activation of caspase 3[23–26]; and (3) via ligation of FAS/CD95, a member of the tumour necrosis factor receptor family, leading to activation of the caspase 8 mediated pathway.

Direct inhibition of granzyme B function

Recently, a novel human intracellular serine protease inhibitor (serpin), called protease inhibitor 9 (PI9), was found to be an efficient inhibitor of granzyme B and to protect cells from granzyme B mediated apoptosis.[27 28] Since then, we have detected PI9 expression in neoplastic cells of several lymphomas, including a small proportion of systemic ALCLs.[29] Importantly, we found that PI9 expression in ALCL correlated with high numbers of tumour infiltrating activated CTLs (unpublished data, 2002), supporting the notion that the presence of many tumour infiltrating activated CTLs results in selection for CTL resistant tumour cells.

Inhibition of downstream apoptosis pathways

The caspase 9 pathway is regulated by many different proteins, including members of the bcl-2 and inhibitor of apoptosis protein family. Together with others investigators, we have recently demonstrated the expression of members of the bcl-2 protein family in ALCL.[30 31] High expression of these proteins was found in particular in ALK negative cases. Its influence on the apoptosis cascade was supported by an inverse correlation between bcl-2 expression and numbers of active caspase 3 positive apoptotic cells.[31] We also found that cases with high numbers of bcl-2 expressing tumour cells usually harboured many activated CTLs (unpublished data, 2002), again suggesting selection for CTL resistant tumour cells.

> "Inhibition of the downstream apoptosis pathway as a putative immune escape mechanism may be especially relevant from a clinical point of view, because inhibition of apoptosis is expected to result in decreased sensitivity to chemotherapy"

CTL induced cell death via ligation of FAS with concomitant activation of the caspase 8 pathway can be inhibited by loss of FAS expression, as has been shown in certain T cell lymphomas,[32] and by expression of cellular FLICE inhibitory protein in the tumour cells, which will interfere with caspase 8 mediated activation of caspase 3.[33] This has not yet been studied in ALCL.

CONCLUDING REMARKS

Although it has not yet been confirmed that ALCLs contain tumour specific activated CTLs, it is probable that the above mentioned immune escape mechanisms play some role in the pathogenesis of ALCL. Inhibition of the downstream apoptosis pathway as a putative immune escape mechanism may be especially relevant from a clinical point of view, because inhibition of apoptosis is expected to result in decreased sensitivity to chemotherapy. We recently provided evidence, although indirectly, that inhibition of apoptosis may indeed be a major determinant for a poor clinical outcome in ALK negative patients with ALCL.[31]

J Clin Pathol 2003;**56**:423–425

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