

## Commentary

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### Signals from lymphocytes in colon cancer

The area of tumour immunology is often characterised as one of sustained optimism, yet although this field has enriched our knowledge of basic immunology, little has emerged to benefit the cancer patient. This may now be changing. With understanding of the structural basis of how T cells recognise antigenic peptides in the context of MHC class I molecules and with the genes for several human melanoma antigens recently cloned,<sup>1</sup> there is now a basis for a more rational design of anti-cancer vaccines. A number of clinical vaccine trials have now begun using identified melanoma antigens. Alternative modes of T cell based anti-tumour therapy involve the re-injection of tumour infiltrating lymphocytes (TIL) expanded in vitro with the cytokine interleukin 2 (IL 2) into patients with solid tumours.<sup>2</sup> As in vitro expanded TIL cells have been shown to contain a high frequency of T lymphocytes with the capacity to kill the autologous tumour, it could be asked why they are unable to perform this task in the tumour microenvironment in the patient. A variety of mechanisms have been suggested to account for this, including the presence of factors or cells with immunosuppressive capacity in the tumour or induction of tolerance by tumour cells lacking costimulatory signals.

An interesting correlate to the poor functional performance of TIL cells is presented by Mulder *et al* (see page 113). They have analysed the expression of the T cell receptor associated  $\zeta$  (TCR  $\zeta$ ) and the granzyme B (GrB) molecules in situ by lymphocytes infiltrating normal colonic mucosa and Dukes's stage A and D colorectal carcinomas. These molecules are of crucial importance in triggering and effecting T cell functions, respectively. The key finding in this immunohistochemical study is that the numbers of TCR  $\zeta$  expressing T lymphocytes in Dukes's A and D carcinomas were lower than in normal mucosa of patients without cancer. The cytoplasmic domain of the CD3  $\zeta$  subunit in the T cell receptor complex is involved in signal transduction and subsequently activation of T cells. The authors therefore argue that a defect in the TCR  $\zeta$  chain would interfere with the signal transducing capacity of the TCR  $\zeta$  chain and would severely hamper the function of anti-tumour immune effector cells by uncoupling antigen recognition and effector functions.

This immunohistochemical study of reduced TCR  $\zeta$  expression in TIL of colorectal carcinomas is related to a series of observations indicating alterations of signal transducing molecules in T cells and NK cells in cancer patients and animals with experimental tumours. Mizoguchi *et al*<sup>3</sup> first reported a decrease in CD3  $\zeta$  chain levels, and of the src family protein tyrosine kinases (PTK) p56<sup>lck</sup> and p59<sup>lyn</sup>, in T cells from mice bearing a colorectal carcinoma. Similar changes have been described in T cells from cancer patients with colorectal,<sup>4,6</sup> renal<sup>7</sup> and ovarian<sup>8</sup> carcinomas, and have been correlated with reduced immunological functions.

The immunohistochemical study by Mulder *et al* elegantly confirms and extends several of the features of

earlier work in this field, which has mainly been based on flow cytometric and biochemical analysis. Relative to normal mucosa, Mulder *et al* find that the numbers of TCR  $\zeta$  expressing lymphocytes in tumours were decreased in Dukes's A and D colorectal carcinomas, being lowest in Dukes's D tumours. This adds to previous findings that the intensity of TCR  $\zeta$  expression in peripheral blood lymphocytes of patients with colorectal carcinoma is decreased with more advanced tumour stages, as measured with flow cytometry and biochemical methods.<sup>5,6</sup>

As Mulder *et al* find that the number of TCR  $\zeta$ <sup>+</sup> lymphocytes decreased in Dukes's A carcinomas, their results suggest that TCR  $\zeta$  downregulation in TIL is a relatively early event in the interaction between a tumour and the immune system. In contrast, they describe an increase in the numbers of GrB expressing lymphocytes in the early stage of colorectal tumours, similar to earlier observations by Nakanishi *et al*.<sup>9</sup> The authors infer that as the expression of this molecule involved in the cytolysis of tumour cells requires both TCR triggering and signalling via a costimulatory receptor some immune stimulation still takes place in Dukes's A carcinoma. In Dukes's D carcinomas, however, GrB expressing cells had disappeared from the tumour tissue, which can explain the lack of cytotoxic activity of TIL cells.

The number of TCR  $\zeta$  expressing lymphocytes in tumours was decreased in the tumour relative to normal mucosa, similar to the 'gradient' of  $\zeta$  expression that was previously described moving from the TIL to the normal mucosa and peripheral blood in patients with colorectal carcinomas.<sup>5</sup> The authors speculate that this may point at the involvement of tumour derived factors as the responsible mechanism, and give some evidence in support of this showing that both TCR  $\zeta$  and GrB were rapidly restored during in vitro culture. These tumour derived factors may be secreted by the tumour cells or by tumour infiltrating mononuclear cells, the latter alternative being supported by recent observations that tumour associated macrophages as a result of cocubation with autologous T cells from cancer patients can induce decreased expression of TCR  $\zeta$ .<sup>10</sup>

Regardless of the underlying mechanism, measuring TCR  $\zeta$  expression might provide vital information that can be used to follow up and optimise immunotherapy of cancer patients. Immunohistochemical analysis, for example, of biopsy specimens taken from the site of anti-tumour vaccination, or flow cytometric assays of T lymphocytes from peripheral blood might provide a particularly convenient way of doing this on a routine basis. The possibility that measuring expression of TCR  $\zeta$  will be of prognostic value should be explored in patients with colorectal carcinoma and other types of human tumours. Research aimed at developing drugs that can counteract suppression of anti-tumour activity, normalising the expression of TCR  $\zeta$  and GrB molecules in cancer patients,

and which should be given in combination with active or adoptive immunotherapy, should provide new and promising avenues for the treatment of cancer

ROLF KIESSLING

Professor of Experimental Oncology,  
Department of Microbiology and Tumorbiology,  
Karolinska Institute, Stockholm, Sweden

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