Lymphocyte infiltration in oesophageal carcinoma: lack of correlation with MHC antigens, ICAM-1, and tumour stage and grade

J C Rockett, S J Darnton, J Crocker, H R Matthews, A G Morris

Abstract
Infiltration by T lymphocytes into oesophageal carcinomas was assessed immunohistochemically, total T lymphocyte numbers by staining for CD3 and activated T lymphocytes by staining for CD25. Five squamous carcinomas and seven adenocarcinomas, resected without neoadjuvant treatment, were studied. Computer aided quantitation showed that total numbers of tumour infiltrating CD3 positive cells were highly variable (range 48-1673 cells/mm²). They were located largely in the stromal (87-99-2%) rather than intratumoral regions. Up to 84% of tumour infiltrating T lymphocytes were CD25 positive, although the median figure was 33%. There was no correlation between T lymphocyte infiltration or activation and expression of class I and II histocompatibility antigens, intercellular adhesion molecule-1, tumour stage or grade. These results imply that the local inflammatory response in oesophageal carcinomas is deregulated, which may be a factor contributing to the aggressive nature of the tumours.

Keywords: ICAM-1, lymphocyte infiltration, MHC, oesophageal carcinoma

Cancer of the oesophagus is an aggressive malignancy with a high mortality rate. The two primary types of this neoplasm, namely adenocarcinoma and squamous cell carcinoma, have overall five year survival rates of only 0-8 and 6-3%, respectively. One reason for such poor survival could be the failure of the host immune system to recognise and eliminate the neoplastic cells.

Paul Ehrlich originally postulated that a tumour may be recognised as antigenically foreign by the host. This concept was later refined into the theory of immunological surveillance against cancer. Evidence cited in favour of the theory includes the accumulation of lymphocytes at most tumour sites. The extent of such lymphoid infiltrations has been assessed in various tumours, and has in many instances

although unlikely, could not be completely excluded as there might have been occult bony lesions.

The pathogenesis and nature of LCH are not clearly understood; viruses and cytokines have been proposed as aetiological agents for the abnormal proliferation of the Langerhans cells. However, there is no conclusive evidence to support these hypotheses. There is also debate whether LCH is a reactive or neoplastic lesion. Many LCH lesions, as in the present report, are asymptomatic, solitary and non-aggressive. The occasional association of LCH with malignant neoplasms and inflammatory processes suggest that LCH may develop as an unusual host reaction to these diseases. To date, genetic abnormalities have not been detected in LCH. All these findings support the view that LCH is a reactive condition. However, the aggressive nature of systemic LCH and the demonstration of monoclonality in some cases suggest that the disease may be neoplastic in nature. In this context it is worth noting that monoclonality does not confirm the existence of malignancy; clonality alone does not predict the behaviour of LCH.

Splenial infiltration by systemic LCH is usually discovered at necropsy and is characterised by a diffuse splenic red pulp infiltration by Langerhans cells. In such cases of splenic involvement, patients may present with fatal thrombocytopenia because of hyper-splenism. Indeed, splenomegaly is a poor prognostic factor in patients with LCH. Splenic rupture has also been reported. In contrast, the splenic lesion in this case was solitary, small and asymptomatic, and would probably have been self-limiting. The present case is the first to be reported of solitary LCH in the spleen. As occult LCH is almost certainly under-diagnosed in adults, we await reports of similar cases in future.

be shown to be a favourable prognostic indicator for postoperative survival. The expression of major histocompatibility complex (MHC) class I molecules, which are expressed on nearly all nucleated cells, has been shown to be aberrant to some degree in most carcinomas. The expression of MHC class II and intercellular adhesion molecule (ICAM)-1, normally induced by cytokines secreted by inflammatory cells, has been shown to be both sporadic and heterogeneous in most malignancies. As all three molecules are important in immune surveillance by T lymphocytes, a loss or reduction in their expression may be one mechanism by which neoplastic cells are able to avoid recognition and subsequent eradication.

In this study we have investigated lymphocyte infiltration in oesophageal carcinoma and compared it with the expression of MHC and ICAM-1. Correlation with a number of clinicopathological parameters has also been sought.

**Methods**

Twelve patients (five with squamous carcinoma and seven with adenocarcinoma) with no neo-adjuvant treatment underwent surgical resection. Following resection a sample was taken immediately from the leading edge of the tumour, snap frozen and stored in liquid nitrogen until needed. A further sample of tumour was fixed in 10% formal saline and processed routinely to paraffin wax.

Consecutive 7 μm cryostat sections from the frozen samples were mounted on untreated microscope slides, air dried for 20 minutes, fixed in acetone at −20°C for 20 minutes and then air dried before storage at −80°C. A 3 μm section from a wax block was cut and stained for B cells.

Immunohistochemical staining was carried out using the Vectastain Elite ABC kit (Vector Laboratories, Peterborough, UK). Antibody to CD3 was used to label the total population of infiltrating T lymphocytes, whilst anti-CD25 was used to identify activated lymphocytes. Anti-CD20 was used to detect B cells. Primary antibodies were monoclonal mouse anti-human: CD3 (Becton Dickinson, San Jose, California, USA) diluted 1 in 100 in phosphate buffered saline (PBS), pH 7·4; CD25 (Dako, High Wycombe, UK) diluted 1 in 100; and CD20 (Dako) diluted 1 in 200. Sections were incubated with the primary and secondary anti-CD3 positive and CD25 positive cell counts in tumour sections (including tumour nests and intertumoral stroma)

<table>
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<th>Sample number</th>
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<th>CD25+ count/mm²</th>
<th>CD25%</th>
<th>Type</th>
<th>Stage</th>
<th>Differentiation (grade)</th>
<th>MHC I</th>
<th>MHC II</th>
<th>ICAM-1</th>
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Tumour type: SCC = squamous cell carcinoma; A = adenocarcinoma. Differentiation: grade 2 = moderate; grade 3 = poor. Staining: + = homogeneously positive; +/− = heterogeneously positive; − = negative. N/A = not assessable.
 Stromal CD3+ cell infiltration in an adenocarcinoma. The majority of stained cells are in the stroma (s) between the tumour cell nests. (Original magnification ×100.)

data for the tumours studied. The median number of CD3 positive cells invading the tumours was 508/mm² (range 48–1673/mm²). Statistical analysis showed no significant difference between overall numbers invading squamous cell carcinomas and adenocarcinomas (U = 8, p > 0.05).

In only nine cases was it possible to discern nests of intratumoral neoplastic cells; the remaining three cases were very poorly differentiated with diffuse spread. The numbers of CD3 positive cells invading these nests (median 15/mm², range 6–203/mm²; data not shown) were very low compared with overall tumour infiltration, the majority of the cells being located in the stroma (median 96.3%, range 87.9–99.2%) (figure). Although the numbers of specimens analysed were small, there were statistically higher numbers of CD3 positive cells invading the nests of neoplastic cells in squamous cell carcinomas than adenocarcinomas (U = 0, p = 0.05).

There was no association found between absolute numbers of infiltrating T lymphocytes (CD3 positive cells) and the expression of MHC I, II or ICAM-1 by the tumour cells.

Counts of total CD25 positive cell infiltration in sections from 10 tumours (table) gave a median of 154.5/mm² (range 62–452/mm²). There was no statistical difference between overall numbers of cells invading squamous cell carcinomas and adenocarcinomas (U = 8, p > 0.05). The numbers of CD25 positive cells infiltrating the tumours were significantly lower than the numbers of CD3 positive cells (p < 0.001). Furthermore, the numbers of CD25 positive cells in each specimen did not correlate with the numbers of CD3 positive cells.

Of the nine specimens where intratumoral neoplastic cell nests were discernable, only five showed CD25 positive cell infiltration. Although four of these five were squamous cell carcinomas, this finding was not statistically significant. Overall, numbers varied from 0 to 35/mm², with a median of 2/mm² (data not shown). Again, these numbers represented only a small proportion (median 1–5%, range 0–18%) of the total infiltrating CD25 positive cells.

No association was found between numbers of overall or intratumoral CD25 positive cells and MHC/ICAM-1 expression.

There were no statistically significant correlations between population densities of CD3 positive or CD25 positive cells and clinicopathological parameters.

No B cells were detected within tumour nests or in the intertumoral stroma.

Discussion

T cell counts were made on areas taken from the leading edge of tumours, where there is an intact mucosal barrier and little necrosis. We therefore believe that the infiltrating T cells represent a response to the tumour, rather than a non-specific inflammatory response to ulceration or necrosis.

CD25 (IL-2 receptor) expression has been used as a marker of T lymphocyte activation. Although CD25 is also a marker of macrophage and B lymphocyte activation, the former are morphologically distinct from lymphocytes and were ignored in the counting procedure. No B lymphocytes were present in the counted areas and therefore the CD25 count is a true reflection of the number of activated T cells.

Although overall numbers of infiltrating T lymphocytes were similar in squamous cell carcinomas and adenocarcinomas, there were significantly higher numbers of these cells infiltrating the intratumoral neoplastic cell nests of the former. Furthermore, four of the five tumours containing activated intratumoral T lymphocytes were squamous in origin. These two observations could explain why patients with squamous cell carcinoma survive better than those with adenocarcinoma.

Individual oesophageal tumours elicit varied immune responses, both in terms of absolute numbers of infiltrating T lymphocytes and of activated cells. This observation may simply reflect the overall varied immune status of individual patients, or perhaps, residual numbers of lymphocytes remaining following the peak anti-tumour inflammatory response. The different counts found could suggest, however, that some form of immune suppression is occurring in those cases with low total or activated T lymphocyte counts.

Only a small percentage of the total infiltrating T lymphocytes was activated. One possible explanation is the downregulation (or lack of upregulation) of MHC class I, MHC class II and ICAM-1 expression on the tumour cells. These molecules normally play a central role in stimulating the immune response. We have shown previously that they are expressed aberrantly or not at all by oesophageal carcinomas. The present study finds no correlation between numbers of CD25 positive cells and the MHC/ICAM-1 status of the tumour. Nouri et al recently presented data in testicular tumours in which they found large numbers of activated T lymphocytes in the absence of MHC and ICAM-1. Our work also suggests that total numbers and activation of
T lymphocytes can be independent of tumour MHC/ICAM-1 expression.

In many inflammatory situations, such as allograft rejection, delayed type hypersensitivity responses and various allergic diseases, increased expression of MHC (particularly class II) and ICAM-1 is thought to be related directly to cytokines secreted from the large numbers of activated mononuclear cells reacting to foreign antigens. Inflammatory responses often occur in cancers, but the nature of the stimulus is unclear. In some situations responses to tumour associated antigens may be occurring; in others the response may be due to necrosis or to the breakdown of the mucosal barrier, allowing leakage of luminal antigens. What is clear is that, whatever the nature of the stimulus, there is some form of functional disregulation of the inflammatory response in oesophageal carcinoma (as has also been seen in other carcinomas such as those of the lung and cervix) as MHC/ICAM-1 expression is not related to T lymphocyte numbers or activation. It is possible that the neoplastic cells of both oesophageal squamous cell carcinomas and adenocarcinomas might produce immunosuppressive factors, such as transforming growth factor-β1, which antagonise or inhibit the production or effect of inflammatory cytokines such as interferon-γ. The elucidation of the mechanisms contributing to the dis-regulation of immune cells such as T lymphocytes is an important challenge, the solution of which might permit more effective treatment of these aggressive tumours.

JCR and SJD are funded by the Oesophageal Cancer Research Appeal (OCRA), Birmingham.

We are most grateful to Ms L Billingham of the University of Birmingham CRC Institute for Cancer Studies for assistance with statistical analysis. Miss K Jenner and Mr G Mannion contributed valuable technical and photographic skills, respectively.


Sudden death due to a glial cyst of the pineal gland

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Abstract

Asymptomatic cysts of the pineal gland are found frequently by radiological examination of the brain or at postmortem examination. Symptomatic cysts are rare, and may require surgical intervention. Sudden death due to a cystic lesion of the pineal gland is very rare. A case of a 22 year old man who collapsed and died unexpectedly is reported. Postmortem examination revealed a glial cyst of the pineal gland and evidence of chronic obstructive hydrocephalus. Deaths from colloid cysts and pineal gland cysts are rare, but should be considered where no other cause of death is evident, especially with a history of headaches. Their small size, and their possible rupture on dissection can make them difficult to detect if a careful examination is not undertaken.

Keywords: pineal gland, cyst, postmortem examination.

Sudden death due to a colloid cyst of the third ventricle, though rare, is well recognised in both adults and children. Asymptomatic cystic lesions of the pineal gland are quite common but symptomatic lesions are very rare. We report the sudden death of a young man due to a glial cyst of the pineal gland.

Case report

A 22 year old white man collapsed at a disco. He had been engaged in a punching game with a friend who involved hitting each other in turn in the shoulder area. Having finished the game he collapsed. Vigorous resuscitation was commenced, but this was unsuccessful.

At postmortem examination, no significant external injuries were present. In particular, no bruising or other injury was present in the shoulder or chest area. Internal examination, including full microscopy, did not reveal any abnormality in any organ system other than the...