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Role of bronchoalveolar lavage in the investigation of cell-mediated defence mechanisms against lung cancer

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Little information is available on the applicability and reliability of bronchoalveolar lavage (BAL) in the evaluation of local cellular defence mechanisms against lung cancer (LC). LC is frequently located in the large airways, whilst BAL allows recovery of cells from the alveolar spaces. Important functional differences may exist between cells obtained by BAL and immunologically competent cells isolated after disaggregation of whole lung tissue specimens, thus BAL does not always sample the pulmonary cell population correctly.

Alveolar macrophages (AM) are thought to play an important role in the host defence mechanism against LC by releasing a variety of cytotoxic and cytostatic substances and by functioning as accessory cells for lymphocytes (LY). The importance of AM is demonstrated by the fact that over 90% of cells recovered from the alveoli by BAL and over one third of cells isolated after disaggregation of LC surgical specimens belong to the monocyte macrophage lineage [1]. These were shown to exert specific cytotoxic activity against autologous LC cells but not against non-malignant tumour targets, hence their toxicity could be related to tumour antigen driven specific responses [2].

Investigations into the differentiation of blood monocytes into AM and association to modifications of tumour killing properties have led to conflicting results. AM from smoking and nonsmoking normals and from patients with LC were shown to be more cytotoxic than autologous blood monocytes for various tumour cell lines (including squamous LC) with no differences between study groups [1]. In contrast, Bordignon *et al.* reported

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defective tumouricidal capacity of AM, compared to mononuclear phagocytes recovered from other anatomical sites (including peripheral blood). Heterogeneity of experimental models may partly explain these contrasts. Mononuclear phagocytes can kill tumour targets by both oxygen dependent and oxygen independent pathways and several different toxic substances may play a role in these mechanisms. Oxygen dependent killing by mononuclear phagocytes is due to release of toxic oxidant species, *e.g.* hydrogen peroxide, superoxide anion and singlet oxygen, in response to particle ingestion or membrane perturbation in the "respiratory bursts". Although stimulated AM undergo a typical respiratory burst, they are unable to kill tumour targets that are sensitive to oxidants and that are killed by peripheral blood monocytes [3]. This may be due to the differentiation related loss of myeloperoxidase, an enzyme known for its ability to enhance the toxicity of reactive oxygen species for tumour cells. Thus, it seems that oxidants might play a minor role in macrophage anti-tumour activity in the alveolar spaces. It is not clear whether other pulmonary mononuclear phagocytes, *e.g.* bronchial, interstitial, intravascular and pleural macrophages, behave like AM or whether they resemble common ancestral cells, *i.e.* blood monocytes.

Oxygen independent killing is mediated by the release of many anti-tumour effector molecules, including proteinases, the complement breakdown product C3a and tumour cytotoxic and cytostatic protein factors. Amongst the latter, tumour necrosis factor (TNF) is of interest. It is a cytokine produced by mononuclear phagocytes and originally recognized as the mediator of lipopolysaccharide (LPS)-induced necrosis of murine sarcomas. It exerts a variety of effects on non-malignant targets and is an important mediator of inflammation. LPS-stimulated AM release comparable levels of TNF to autologous blood monocytes, indicating that the ability to release this monokine is not lost during differentiation within the lungs [4]. Preliminary data suggest that TNF exerts very low levels of cytotoxicity upon LC cell lines *in vitro*, but may reduce the rate of proliferation of these cells, an effect enhanced by the addition of interferons. Since TNF up-regulates the release of other important mediators, *e.g.* reactive oxygen intermediates and interleukin 1, its role in AM-mediated defence against LC looks complex and presumably involves other effector cells. The relevance of these phenomena to the onset and spread of the disease needs elucidation. Other substances take part in AM-mediated anti-tumour activity and AM may function as accessory cells for LY.

The anti-tumour activity of LY is mediated by different mechanisms of tumour cell cytotoxicity and by the release of lymphokines that may activate other effector cells, such as AM. Due to the low yield of LY from BAL few studies have been performed on LY-mediated local immune defence in LC and most have investigated natural killer (NK) activity. NK cells are non-adherent lymphoid cells with the ability to lyze *in vitro* many tumour targets without prior sensitization and without restriction by major histocompatibility antigens. It has been suggested that NK play a role *in vivo* as one of the first lines of resistance against malignancies because, unlike other cytolytic lymphoid cells, they rapidly and spontaneously kill tumour cell targets.

Robinson *et al.* demonstrated that lymphoid cells with the morphological and phenotypical characteristics of NK cells are present in the alveolar spaces and can be recovered by BAL. However, these cells are functionally inactive, possibly due to the inhibitory effect of unidentified products released by AM. In normal conditions, NK cells isolated from whole lung tissue have been shown to exert levels of cytotoxicity comparable to peripheral NK cells. Thus, the pulmonary NK inefficiency is compartmentalized to the alveolar spaces, the interstitial NK cells being actively cytotoxic [5]. In patients with LC, the activity of lung interstitial NK cells is markedly reduced as compared to normals; this reduction was compartmentalized to the lungs since high levels of peripheral blood NK activity were shown in both groups [5]. The suppression of interstitial NK activity was directly related to the total numbers of macrophages recovered from the lung tissue, suggesting that the accumulation of mononuclear phagocytes within the lung could account for the defective lung NK activity. Since cigarette smoking (the major risk factor for LC) is associated with increased numbers of AM, its tumour-promoting activity could be partly due to inhibition of lung NK activity. In contrast, Pitchenik *et al.* demonstrated increased NK activity levels in BAL fluid from LC patients. This increase was thought to be associated with local stimulation by interleukin 2 on NK cells, as indicated by increased levels of this lymphokine in BAL fluid.

Further studies are required to understand these discrepancies and the role of AM and pulmonary LY and NK cells in defence mechanisms. The need for information in this field has increased with the recent availability of powerful biological response modifiers (*e.g.* interferons and interleukin 2) that may change the therapeutic approach to LC. BAL is a powerful research tool in providing this information.

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