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Maturation of dendritic cells in the presence of living, apoptotic and necrotic tumour cells derived from squamous cell carcinoma of head and neck

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KEYWORDS

Head and neck squamous cell carcinoma;
Tumour antigen;
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T lymphocytes;
Cytokines

Summary Dendritic cells (DC) have been recently used as vaccines for stimulation of tumour-specific immunity in various types of cancer. Since data about interactions of DC with tumour cells derived from head and neck cancer are not available, in our study we investigated the effects of head and neck squamous cell carcinoma (HNSCC) cell lines on the maturation of DC. We found that immature DC efficiently internalise necrotic cells, but not living and apoptotic tumour cells. Although apoptotic cells induced a partial maturation of DC, they were not able to stimulate the secretion of IL-12. In contrast, necrotic tumour cell preparations from all three HNSCC cell lines induced the mature phenotype and IL-12 production by DC. Moreover, necrotic cells synergistically augmented stimulatory effects of monocyte-conditioned medium on the maturation of DC. Thus, DC-based vaccination utilizing necrotic tumour cells as a source of tumour antigens, even in combination with inflammatory stimulus, seems to be a suitable strategy for adjuvant immunotherapy in HNSCC.
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Abbreviations: HNSCC; head and neck squamous cell carcinoma; HNC; head and neck cancer; MCM; monocytes-conditioned medium; PBMC; peripheral blood mononuclear cells; DC; dendritic cells; imDC; immature DC; maDC; mature DC; APC; antigen-presenting cells; HSP; heat shock proteins; IL; interleukine; Th; helper T lymphocytes; Treg; regulatory T lymphocytes; MR; mannose receptor

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Introduction

Integration of immunotherapy into the armamentarium for the management of cancer has substantial promise and represents one of the challenges of this decade. Continued research in the immunobiology of solid tumours has yielded hope that immune defence mechanisms may contribute to the therapy of head and neck cancer (HNC). Although there have been significant advances in the use of therapeutic antibodies, cytokine therapy and dendritic cell (DC) vaccines in other types of epithelial cancer, the knowledge about interactions between DC and tumour cells in head and neck squamous cell carcinoma (HNSCC) is merely limited.[1,2]

DC represent a discrete leukocyte population of professional antigen-presenting cells (APC) with an extraordinary capacity for initiating primary T lymphocyte responses.[3,4] In their immature state, DC efficiently and continuously sample the antigenic content of their environment by high-volume fluid phase macropinocytosis, receptor-mediated endocytosis or by phagocytosis of infected, apoptotic or necrotic cells. Upon receipt of a maturation signal, DC downregulate their antigen-uptake machineries, become differentiated to activate T cells and upregulate the adhesion and costimulatory molecules. This maturation process can be triggered by multiple stimuli, including lipopolysaccharide, bacteria, viruses, monocyte-conditioned medium, CpG oligonucleotides and cell signalling molecules.[5]

Combined chemoradiotherapy generates huge amounts of killed or damaged tumour cells in treated cancer patients. Such dead and dying tumour cells (necrotic and apoptotic tumour cells, respectively) have been shown previously to be a source of tumour-associated antigens for presentation by DC. However, there is a disagreement in the field with respect to the ability of apoptotic and necrotic tumour cells to induce the DC maturation process and anti-tumour immunity.[6] Earlier reports have demonstrated that DC, which phagocytosed apoptotic cells, are able to initiate an anti-tumour immune response.[7] It was shown later that necrotic, but not apoptotic cells induce the maturation of DC.[8] They elicit protective immunity and heat shock proteins (HSP), which are released from necrotic cells only, were implicated to induce the maturation of DC.[9]

In the present study, we investigated the effects of living, apoptotic and necrotic HNSCC cells on the maturation and cytokine secretion from DC.

Materials and methods

HNSCC cell lines

HNSCC cell lines RPMI-2650, BHY and CAL-27 were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). RPMI-2650 is squamous cell carcinoma (SCC) of nasal septum, BHY was obtained from a patient with SCC of oral floor and CAL-27 was derived from tongue SCC. All the cell lines were propagated in the RPMI/10%FCS medium.

Cell preparation and culture conditions

Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood of healthy donors using centrifugation on a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient, as described previously.[10] Monocytes were separated from PBMC by adherence on gelatine-coated petri dishes. After 40 min at 37 °C, non-adherent cells were removed by aspiration, and dishes were washed with warm RPMI medium. Adherent cells were detached with RPMI supplemented with 5 mM EDTA. To obtain monocytes-derived dendritic cells (DC), monocytes were washed and cultivated in RPMI/10% FCS supplemented with 1000 U/ml inteleukin-4 (IL-4) and 1000 U/ml GM-CSF at 10^6 cell/ml density in six-wells plates (Costar, Cambridge, MA). 1000 U/ml IL-4 and 1000 U/ml GM-CSF were added after 2 days. On day 5, cells were collected, washed, and used for cocultures with tumour cells. DC cultivated for further 2 days in the presence of fresh RPMI/10% FCS supplemented with IL-4 and GM-CSF without any stimulation (immature DC—imDC), stimulated with 10 ng/ml of LPS (mature DC—maDC) or with 20% (v/v) of monocyte-conditioned medium (MCM) were used as controls, respectively.

Co-culture of tumour cell lines with DC

HNSCC cells were collected using trypsin/EDTA and subsequently washed twice with PBS. For induction of apoptosis, HNSCC cells in suspension were exposed to γ-irradiation (Cs^{137} source). More

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