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SCIENCE DIRECT
Immunobiology 211 (2006) 179–189
Immunobiology
www.elsevier.de/imbio

Preparation of native and amplified tumour RNA for dendritic cell transfection and generation of in vitro anti-tumour CTL responses

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Received 28 April 2005; accepted 30 August 2005

Abstract

Recent research indicates that dendritic cells transfected with RNA-encoded tumour-associated antigens (TAA) can generate potent anti-tumour immune responses in vitro and in vivo. RNA is an important source of TAA, but its relatively unstable nature, in addition to often limited availability of tumour tissue, may represent a considerable obstacle for its use. Our first goal was to establish an efficient protocol for the preparation of high quality total RNA from tumour samples. This should then be used as such or be pre-amplified for DC transfection. Therefore native total RNA was prepared from stabilised tissue samples obtained from liver metastases of colon cancer using either solution- or silicagel-based protocols for RNA isolation. The first isolation protocol yielded higher amounts of total RNA, but with lower purity as compared to the second one. No degradation of RNA was observed regardless of the protocol used. Subsequently, we focused on the amplification of mRNA. The fidelity of the amplified mRNA was confirmed by RT-PCR for glyceraldehyde-3-phosphate-dehydrogenase (GADPH) and carcinoembryonic antigen (CEA) coding sequences. We found no differences in the induction of CEA-specific CTL responses between native and amplified RNA-transfected DCs. Additionally, we tested the induction of CTL responses and found that DCs transfected with amplified mRNA originating from either tumour tissue or a cell line were able to induce strong anti-tumour CTL responses in vitro. They were comparable to those induced by native total RNA-transfected DCs. Our results therefore indicate that the amplified mRNA is equivalent to the native one in the induction of anti-tumour CTL responses and can be used for generation of RNA-transfected DCs.

Keywords: CEA; CTL responses; Dendritic cells; mRNA amplification; RNA isolation

Introduction

Dendritic cells (DCs) are the most potent antigen presenting cells (APCs). They are crucial for efficient immune responses against pathogens as well as for the regulation and maintenance of immunological tolerance (Banchereau and Steinman, 1998). Given their immunological potency, DCs are considered a promising

Abbreviations: CEA, carcinoembryonic antigen; CTL, cytotoxic T lymphocyte(s); DC, dendritic cell(s); GADPH, glyceraldehyde-3-phosphate-dehydrogenase; PBMC, peripheral blood mononuclear cell(s); TAA, tumour-associated antigens

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doi:10.1016/j.imbio.2005.08.008

vector for immunotherapy of various cancers (reviewed in Cranmer et al., 2004). DCs are able to process tumour-associated antigens (TAA) and to present them to naïve T cells, concomitantly assuring efficient costimulatory activity through their abundantly expressed costimulatory molecules (Nestle et al., 2001). Therefore, they are the most important APCs involved in primary anti-tumour T-cell immune responses (Schuler and Steinman, 1997; Steinman and Dhodapkar, 2001). It is believed that in vitro cultured DCs, properly loaded with relevant TAA and supplied with the necessary activation signals, are able to break T-cell tolerance to tumours and thus induce functional cytotoxic T-cell (CTL) anti-tumour immune responses in cancer patients. For this reason, the delivery of TAA in their appropriate form to DCs is a very important step in anti-tumour DC vaccine preparation.

TAA can be delivered to DCs in multiple forms, mainly as particular peptides (Nestle et al., 1998; Thurner et al., 1999), proteins (Timmerman et al., 2002) or entire contents of tumour cells (Nestle et al., 1998; Thumann et al., 2003). Another approach is a gene-based delivery of TAA, which includes DNA (Larregina et al., 2004) and recently RNA, either as a whole RNA isolated from tumour cells or as a defined TAA encoding mRNA. The efficiency of RNA-loaded DCs has been demonstrated in multiple in vivo murine models and in vitro studies (reviewed in Gilboa and Vieweg, 2004). The findings of these studies served as a basis for the initiation of preclinical and clinical studies in humans.

As a source of TAA, RNA has certain advantages over proteins or TAA encoding DNA. Compared to proteins, RNA can be rather easily produced and allows template modification, such as the inclusion of defined sequences that can, for example, improve antigen processing and presentation (Bonehill et al., 2004). In contrast to DNA, there is no risk of mRNA integration into the host genome (Lu et al., 1994). Apart from the viral vector-based DNA transfer to DCs, which is almost entirely limited to experimental studies, the non-viral DNA transduction methods are far less efficient and require complicated transcriptional mechanisms for the proper expression of delivered genetic material (Ponsaerts et al., 2003). Perhaps the biggest advantage of the RNA-based approach is that it can be used in patients with a small tumour burden, which is exactly where the immunotherapy with DCs is supposed to be the most effective.

The RNA encoding defined TAA can be rapidly generated in an in vitro RT-PCR reaction coupled to a transcription reaction using appropriate primers (Boczkowski et al., 1996; Kalady et al., 2002). There is growing evidence that there may be a significant advantage in inducing immune response to a broad spectrum of the patient's own TAA, rather than targeting a defined, single antigen (Boczkowski et al., 1996). The primary advantage of DCs loaded with undefined TAA is their ability to induce immune responses against a wide variety of potential tumour antigens, both MHC class I- and class II-restricted. Additionally, in contrast to the induction of a limited T-cell clone repertoire following the usage of defined TAA, the vaccination with DCs loaded with numerous undefined TAA allows the generation of a broader assortment of responding T-cell clones (Berard et al., 2000). Thus the tumour's escape from immune surveillance due to modulated expression of its TAA array can thereby be prevented.

Taking all this into account, the protocol for in vitro amplification of whole mRNA has been developed by Boczkowski et al. (2000). The procedure based on PCR amplification of the whole cDNA repertoire has further been modified to such a degree that mRNA isolated from only few tumour cells can be amplified to amounts sufficient for DC vaccine preparation (Grunebach et al., 2003; Heiser et al., 2001).

Although RNA, which is a relatively unstable molecule, has been recognised as an important source of TAA, reports on collection of tumour material and subsequent isolation of RNA are scarce in the literature. We have therefore decided to establish an efficient protocol for handling and manipulating RNA isolated from tumour samples in order to achieve optimal yields and quality of RNA to be later transfected to DCs. In the first part of our study, we focused on RNA isolation from tumour samples that were obtained after therapeutic hepatectomy. In addition, we established a modified protocol for mRNA amplification that is based on the original procedure published by Boczkowski et al. (2000). The modifications were mostly done in the primer design and in the PCR reaction parameters in order to improve the yield of the amplified mRNA. In the second part, we evaluated the amplified RNA with regard to the presence of coding sequences for a housekeeping gene glyceraldehyde-3-phosphate-dehydrogenase (GADPH) and a tumour-associated carcinoembryonic antigen (CEA). As the amplified RNA should be comparable to native RNA in terms of DC transfection efficiency and vaccine preparation, we also decided to evaluate the induction of CTL responses, comparing total and amplified tumour RNA-transfected DCs as stimulators of responding T cells.

Materials and methods

Tissue acquisition

Tumour material was collected with the approval of the National Ethical Committee after standard