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Immunization with apoptotic pseudovirus transduced cells induces both cellular and humoral responses: A proof of concept study in macaques

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ABSTRACT

Dendritic cells are able to present viral antigens to T-cells after uptake of apoptotic bodies derived from virus-infected cells. Immunization with virus-infected apoptotic cells was previously shown to induce HIV-specific immune responses in mice. Here we evaluate the safety and immunogenicity of immunization with activated apoptotic cells in non-human primates using autologous T-cells infected with replication defective VSV pseudotyped $SIV_{mac239}\Delta env$. Animals were immunized with γ-irradiated activated T-cells carrying the VSV*env*$SIV_{mac239}\Delta env$ pseudovirus. SIV Gag-specific cellular immune responses were induced as early as two weeks after the first immunization eliciting a biased IFN-γ and IL-2 response. In addition, induction of SIV Gag-specific antibody responses and high titer neutralizing activity against the SIV pseudovirus harboring a VSV-*env* were detected after two immunizations. The vaccinated group and a control group of Chinese rhesus macaques were intravenously challenged with pathogenic SIV_{mac251}. All animals became infected, but SIV-replication was effectively suppressed (below 100 copies/ml) in several animals in both groups. However the group immunized with apoptotic cells revealed better preservation of the gut $CD4^+$ T-cell compartment. Viral control was inversely correlated with an early (4 weeks) but transient increase in the percentage of $Ki67^+CD4^+$ peripheral blood T-cells (Spearman −0.73). We here show that immunizations with activated apoptotic lymphocytes expressing transduced SIV genes result in induction of both cellular and humoral immune responses. This study provides evidence for an immunological principle demonstrating that certain apoptotic cells can be considered as carriers of antigens directing immune responses in macaques.

1. Introduction

There is increasing evidence that intracellular pathogens such as influenza, vaccinia, HIV-1, mycobacterium tuberculosis, EBV or CMV will eventually induce apoptotic cell death in their target cells [1–7]. Foreign antigens expressed in these apoptotic bodies are processed for MHC class I presentation pathways by cross-presentation resulting in the induction of antigen-specific $CD8^+$ T-cells [8]. A recent study suggests that antigen presentation following uptake of apoptotic cells may be more efficient compared to antigen presentation following exposure to inactivated HIV-1 [9]. We have previously demonstrated that EBV, HIV-1, and oncogenic DNA present in apoptotic bodies is transferred, translated and subsequently expressed by antigen-presenting cells [10–12]. In addition to antigen presentation on MHC class I molecules, uptake of material from apoptotic cells also leads to antigen presentation on MHC class II molecules. Uptake of for example, canary pox virus infected apoptotic cells gives rise to antigen presentation on both MHC class I and class II molecules with subsequent activation of both $CD4^+$ and $CD8^+$ T-cell responses [13–16].

However, not all apoptotic cells are immunogenic and there is ample evidence showing dampening of immune activation and induction of regulatory cytokines such as IL-10 and TGFβ after uptake of apoptotic cells [17]. Efforts are underway to utilize apoptotic cells to down-modulate immune responses in settings such as transplantation [18]. We previously showed that the precise activation status of T-cells prior to apoptosis induction dictates whether dendritic cells will be induced to mature, and significantly

Abbreviations: LPS, lipopolysacharide; MuLV, murine leukemia virus; GFP, green fluorescent protein; PBMCs, peripheral blood mononuclear cells; mAb, monoclonal antibody; wks, weeks; AUC, area under curve; SFU, spot forming units; imm, immunizations; NC, non-controllers; C, controllers.

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enhance activation of immune responses [19,20]. Apoptotic mitogen activated T-cells induced dendritic cell maturation including pro-inflammatory cytokine responses such as TNF-α, IL-6 and MIP-1β. In contrast, resting apoptotic T-cells did neither induce dendritic cell maturation nor pro-inflammatory cytokine responses [19]. Further evidence demonstrating that the regulation of the immune system can be influenced by the state and nature of the cells undergoing cell death was exemplified by priming of Th_{17} cells using apoptotic lipopolysacharide (LPS) activated B-cells. Conversely, uptake of apoptotic cells in the absence of microbial signals induced differentiation of regulatory T-cells in mice [21].

Several vectors under development are taken up by their respective target cell *in vivo*, leading to antigen production encoded by the vector [22]. The target cells may subsequently present antigen directly to the immune system in the context of MHC molecules. In addition, antigen presentation may also occur after induction of cell death in the producer cell, whereby neighboring professional antigen presenting cells take up the dying cell, process the material and present the antigens [8,15,16,23–25]. Hence, the mechanism of cross-presentation on MHC class I molecules to $CD8^+$ T-cells as well as presentation on MHC class II molecules to $CD4^+$ T-cells may provide an important mechanism for induction of immune responses to infected cells, cancer cells and after immunization with vaccine vectors capable of inducing cell death [15,16,23–29]. Analyses of the prerequisites for induction of immunogenic *versus* tolerogenic cell death and the type of immune responses induced after cell death in different cells are therefore required in order to better understand and tailor immunization strategies utilizing apoptotic cell-mediated immune responses. The overall aim of the present study was to investigate induction of immune responses after immunization with non-replicating SIV genes expressed in apoptotic cells, as a proof of concept. We reveal the safety and immunogenicity of this approach and examine potential vaccine efficacy in a macaque model. The results presented here demonstrate that activated apoptotic T-cells containing viral genes are indeed immunogenic and capable of inducing both SIV-specific T-cells and antibody responses.

2. Materials and methods

2.1. Ethics statement

Chinese origin rhesus monkeys (Macaca mulatta) were housed at the Biomedical Primate Research Center, Rijswijk, The Netherlands, according to international guidelines for non-human primate care and use (The European Council Directive 86/609/EEC, and Convention ETS 123, including the revised Appendix A). The Institutional Animals Care and Use Committee; DEC-BPRC, approved the study protocols (permit number DEC#614). During the experiment the animals were housed separately in cages, because of the risk of SIV transmission. Animals were checked twice daily for general condition, appetite and stool. Animals were sedated with ketamin before bleedings and test substance administration. The details of animal welfare and steps taken to ameliorate suffering were in accordance with the recommendations of the Weatherall report, "The use of non-human primates in research".

2.2. Animals, immunizations and viral challenge

Mature captive-bred Chinese origin rhesus monkeys (Macaca mulatta) were used in this study. The rhesus macaques were negative for antibodies to SIV-1, simian type D retrovirus and simian T-cell lymphotropic virus at the initiation of the study. Behavior and clinical symptoms (including appetite) were observed daily during the whole experiment by biotechnical personnel. Body weight and body temperature were measured before the start of the experiment and each time the animals were sedated for test substance administration and/or blood sample collection, immediately after sedation of each individual animal. Local reactions at injection sites were checked visually and skin thickness was measured (skin thickness meter, Mitotoyo Japan) at days 1, 2, and 4 after each immunization. Reactions were quantified and recorded (redness, swelling, indurations).

Six rhesus macaques (9–10 years old) were immunized at wks 0, 8, and 17 in a dose escalation regimen with approximately $50–200 \times 10^6$ apoptotic cells of autologous activated SIVΔenv infected PBMCs. Cells were administered by intradermal injections (approximately 20×10^6 cells were resuspended in 100 μl phosphate buffered saline (PBS) and used for each injection site; maximum 10 sites close to inguinal or axillar lymph nodes). A control group of five rhesus macaques were included in the challenge study. At wk 40, (24 wks after the last immunization), animals were challenged with 25 $TCID_{50}$ (5.2 log viral copies) of the heterologous pathogenic cell-free SIV_{mac251} stock propagated on concanavalin A stimulated human PBMCs (kindly provided by Dr. N. Letvin, Beth Israel Deaconess Medical Center (BIDMC), Boston, MA, USA) by the intravenous route (1 ml per animal).

2.3. VSV pseudotyped SIVΔenv vaccine construction and preparations

The SIVΔ*env* vector construct was kindly provided by Dr. K. Uberla (Department of Molecular and Medical Virology, Ruhr University Bochum, Bochum, Germany). This *env* deletion mutant of SIV contains the *gfp* reporter gene in place of *nef* (Δenv-GFP) and was derived from pBRmac239 and generated as described by Schnell et al. [30]. G-VSV pseudotyped SIVΔ*env* vectors were generated by co-transfection of 293T/17 cells with SIVΔEnv-GFP and an expression plasmid for the G protein of VSV. Virus titers were evaluated on TZM-bl indicator cells using a luciferase assay, C8166 indicator cells using FACS analysis to detect GFP expression, and CD3 stimulated rhesus PBMC using FACS analysis to detect GFP expression and intracellular SIV Gag p24 protein expression [10,31]. For the luciferase reporter gene assay, freshly trypsinized JC53-BL (TZM-bl) cells (10.000 cells in 100 μl medium containing 37.5 μg/ml DEAE-dextran) were plated in 96 well flat bottom culture plates and pseudovirus was added in a dilution range. After a 48 h culture period, 150 μl of culture medium was removed from each well and 100 μl of Britelite plus reagent (PerkinElmer, Boston MA USA) was added to the cells. After 2 min incubation at room temperature, 150 μl of cell lysate was transferred to 96 well black/white solid plates for measurement of luminescence using a Victor light luminometer (PerkinElmer). Only those viral stocks that gave > 5% transduction of CD3 stimulated PBMC, when 100 μl of the stock was added to 1×10^6 PBMC, was used for tranduction of macaque PBMC.

PBMC isolated from peripheral blood were used for generation of autologous SIVΔ*env* infected apoptotic cells. PBMC (2×10^6/ml) were stimulated with anti-CD3 mAb (clone SP34, BD,) (10 μg/ml were used for coating of plates) and anti-CD28 mAb (clone L293, BD) (1 μg/ml) in RPMI1640/10% FCS. After over night culture the cells were harvested and plated on fibronectin coated 24 well plates at 1×10^6 cells per well. For the coating, fibronectin (Retronectin CH296, Takara) was diluted in PBS at a concentration 18 μg/ml and 0.5 ml was added per well, followed by 1 h incubation at room temperature. Subsequently, wells were washed three times and the CD3/CD28 blasts were cultured in IL-2 (20 U/ml) containing medium in the presence G-VSV pseudotyped SIVΔ*env* virus. After two days incubation at 37 °C the transduction efficiency was determined by measuring GFP expression in combination with intracellular SIV Gag p27 staining. To this end a small sample was