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Dissecting antigen processing and presentation routes in dermal vaccination strategies

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ABSTRACT

The skin is an attractive site for vaccination due to its accessibility and presence of immune cells surveilling this barrier. However, knowledge of antigen processing and presentation upon dermal vaccination is sparse. In this study we determined antigen processing routes that lead to $CD8^+$ T cell activation following dermal DNA tattoo immunization, exploiting a model antigen that contains an immunoproteasome-dependent epitope. In agreement with earlier reports, we found that DNA tattoo immunization of wild type (WT) mice triggered vigorous responses to the immunoproteasome-dependent model epitope, whereas gene-deficient mice lacking the immunoproteasome subunits β5i/LMP7 and β2i/MECL1 failed to respond. Unexpectedly, dermal immunization both of irradiated bone marrow (BM) reconstituted mice in which the BM transplant was of WT origin, and of WT mice transplanted with immunoproteasome subunit-deficient BM induced a $CD8^+$ T cell response to the immunoproteasome-dependent epitope, implying that both BM and host-derived cells contributed to processing of delivered model antigen. Depletion of radiation-resistant Langerhans cells (LC) from chimeric mice did not diminish tattoo-immunization induced $CD8^+$ T cell responses in most mice, illustrating that LC were not responsible for antigen processing and $CD8^+$ T cell priming in tattoo-immunized hosts. We conclude that both BM and non-BM-derived cells contribute to processing and cross-presentation of antigens delivered by dermal DNA tattoo immunization.

1. Introduction

The earliest successful vaccination against smallpox was accomplished by cutaneous vaccination. Nowadays most vaccines are administered intramuscularly, but the skin remains a very attractive target for vaccination, because of its accessibility and possibilities for lower antigen doses. Currently, a number of cutaneous delivery methods are being tested, including different types of microneedles and tattoo immunization. While these methods have been demonstrated to induce both humoral and cellular responses, the underlying mechanisms contributing to cellular immune activation have only partially been explored. Vaccination-induced priming of $CD8^+$ T cell responses requires the cross-presentation of intradermally delivered antigens by professional antigen presenting cells (pAPC), to $CD8^+$ T cells in the draining lymph nodes. Different studies have defined a variety of pAPC subsets as responsible for the interaction with vaccine antigen-specific $CD8^+$ T cells, including dendritic cells (DC) residing in the lymph nodes, $langerin^+$ dermal DC, and Langerhans cells (LC), although LC may either have a stimulatory or inhibitory role [1–4]. Moreover, while induced $CD8^+$ T cell responses are primed by either of these DC subsets, it remains unclear whether these DC process the epitopes they present, or acquire them from other, non-dendritic, cells.

The epitopes, presented on (p)APC to $CD8^+$ T cells, are processed mainly by proteasomes, which are multi-catalytic enzyme complexes present in the cellular cytosol and nucleus. Proteasome' catalytic activity is displayed by three subunits, β1, β2 and β5, present in the inner two β rings of the 20S proteasome catalytic core particle. Exposure of cells to inflammatory cytokines induces the

Abbreviations: DTR, diphtheria toxin receptor (DTR); KO, knock out; LC, Langerhans cell; KI, knock in; WT, wild type.

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expression of three facultative catalytic sites, β1i/LMP2, β2i/MECL1 and β5i/LMP7, which replace their constitutively expressed homologues in newly assembled proteasomes, leading to the formation of intermediate-type proteasomes and immunoproteasomes [5]. Depending on the presence of either the inducible subunits or their constitutive homologues, proteasomes display different catalytic pocket conformations and peptide transport dynamics [6], which quantitatively alters the pool of peptides produced by proteasomes [7–9].

In contrast to most cell types, pAPCs express the proteasome immunosubunits continuously and contain relatively high quantities of immunoproteasomes. In previous studies using β2i/MECL1 and β5i/LMP7 double gene-deficient (β2i/MECL1$^{-/-}$β5i/LMP7$^{-/-}$) KO mice [10], we showed that priming of CD8$^+$ T cell responses specific for an adenovirus model antigen-derived epitope, E1B$_{192\text{-}200}$, required immunoproteasome-mediated antigen processing. CD8$^+$ T cell responses to a second epitope derived from this antigen, E1A$_{234\text{-}243}$, were unaffected by the absence of immunosubunit expression in these mice. We decided to use this model system to determine antigen processing and presentation routes that lead to the priming of the CD8$^+$ T cell response after dermal DNA tattoo immunization [11]. Using BM chimeric mice, composed of WT -, CD207-diptheria toxin receptor knock in (KI) – and β2i/MECL1$^{-/-}$-β5i/LMP7$^{-/-}$ (KO) recipients, reconstituted with WT – or KO BM, we show that both BM- and non-BM-derived cells contribute to the processing of pAPC-presented, dermally delivered vaccine antigen, and that radiation-resistant LC are not responsible for the CD8$^+$ T cell activation.

2. Material and methods

2.1. DNA vaccine

To generate the E1 DNA vaccine, the sequences coding for the Adenovirus early-1-region (E1) derived epitopes E1A$_{234\text{-}243}$ (SGPSNTPPEI) and E1B$_{192\text{-}200}$ (VNIRNCCYI), each flanked by their natural flanking sequences (encoding 15 amino acids, both N- and C-terminally) [10], were inserted into the pVAX1 vector (Invitrogen), 3′ of and in frame with a tetanus toxin fragment C domain 1 (TTFC)-encoding region [12,13].

2.2. Mice and dermal DNA tattoo immunization

For construction of chimeric mice, bone marrow was flushed from the femurs of donor mice, depleted of mature B and T cells by incubation with a mixture of 10 μg/mL anti-mouse CD4 (clone GK1.5; made in house), CD8 (clone YTS-169; made in house), CD3 (12A2 clone; made in house) and CD19 (clone ID3; made in house), and subsequent incubation with guinea pig complement 4.5 μg/mL for 30 min (Invitrogen). Recipient mice were irradiated with 9 Gy as a single dose from an X-ray irradiator and reconstituted with 10^7 BM cells. They were allowed to reconstitute for 6 weeks. C57BL/6 J mice were purchased from Charles River, B6.129S2-CD207$^{tm3(DTR/GFP)Mal}$/J from Jackson and B6.SJL mice and β2i/MECL1$^{-/-}$β5i/LMP7$^{-/-}$ mice were bred in the animal facility of Utrecht University. The efficacy of reconstitution in mixed bone marrow chimeric mice was evaluated by staining splenocytes with anti-mouse CD11c-APC (clone N418; Biolegend), MHC-II-PE (clone M5/114.15.2; Biolegend), CD45.1-PerCPcy5.5 (clone A20; Biolegend) and CD45.2-FITC (clone 104; Biolegend) and percentages of host-derived DC was measured by FACS (Supplementary Fig. 1).

All mice were immunized at day 0, 3 and 6 with 15 μl cDNA (2 μg/μl) in TE buffer with a 9-needle bar mounted on a tattoo rotary device (Cheyenne) on 100 Hz, at 1 mm depth for 1 min [11]. All animal experiments were approved by the Animal Ethics Committee from Utrecht University (DEC 2013.II.07.084).

2.3. LC Depletion

Depletion of LC in bone marrow chimeric mice in which B6.129S2-Cd207$^{tm3(DTR/GFP)Mal}$/J mice had been reconstituted with B6.SJL bone marrow or β2i/MECL1$^{-/-}$β5i/LMP7$^{-/-}$ BM, was performed by *i.p.* injection of 7.5 ng/gr body weight diphtheria toxin (Sigma) in PBS at day −2, 0 and 6. Efficiency of depletion was measured by FACS analysis at day 0 (Supplementary Fig. 3).

2.4. rLM-E1 Infection

Recombinant *L. monocytogenes* rLM-E1 was grown in brain-heart infusion medium (BD Biosciences) supplemented with 250 μg/ml spectinomycin and harvested while in log phase. Mice were inoculated *i.v.* in the tail vein with a sub-lethal dose of 5000 CFU in 100 μl PBS.

2.5. Analysis of specific CD8$^+$ T cell responses

2.5.1. Intracellular cytokine staining (ICS)

Donor derived CD8$^+$ T cell responses were quantified as reported [8]. Briefly, 2.5×10^6 erythrocyte depleted splenocytes were incubated with or without 1 μg/ml synthetic E1B$_{192\text{-}200}$ VNIRNCCYI or E1A$_{234\text{-}243}$ SGPSNTPPEI for 6 h at 37 °C in RPMI 1640 medium supplemented with 10% FCS-HI (Lonza), 2 mM L-glutamine, 30 μM 2-mercaptoethanol (Gibco), 10 μM monensin (eBioscience) and penicillin/streptomycin. In case of splenocytes from mice infected with rLM-E1, 50 μg/mL gentamycin (Gibco) was added to the medium as well. Cells were stained with anti-mouse CD45.1-PerCPcy5.5 (clone A20; Biolegend), CD45.2-FITC (clone 104; Biolegend) and CD8-APC (clone 53–6.7; eBioscience) in the presence of anti-mouse CD16/CD32 (clone 2.4G2; made in house), fixed and stained with IFNγ-PE (clone XMG1.2; eBioscience) and analyzed on a FACS Canto II (BD Biosciences) using FlowJo software (Tree Star).

2.5.2. IFNγ ELISPOT

MAIP ELISPOT plates (Millipore) were coated with 2 μg/ml anti-mouse IFNγ (clone AN18; made in house) in PBS overnight at 4 °C. Wells were washed and blocked with RPMI 1640 medium (Life Technologies) containing FCS HI (Lonza). 5×10^5 or 2.5×10^5 erythrocyte depleted splenocytes were plated with or without 1 μg/ml synthetic peptide for 6 h in 1 ml FCS-HI and 2-mercaptoethanol (Gibco) supplemented RPMI at 37 °C. Plates were washed with PBS plus 0.01% tween 20 (PBST), and IFNγ was detected with biotinylated anti-mouse IFNγ (clone XMG1.2; BD), followed by alkaline phosphatase-conjugated streptavidin (Jackson Immuno Research Laboratories), in PBST supplemented with 2% BSA. The assay was developed with the Vector blue substrate kit (Vector Laboratories) and analyzed using an ELISPOT plate reader and scanner (AELVIS).

2.5.3. Statistical analysis

To compare donor-derived responses to individual epitopes between the different groups of mice, epitope specific responses of every mouse were corrected for background IFNγ level as measured in samples incubated without peptide, in both IFNγ- ELISPOT and IFNγ ICS. Differences in CD8$^+$ T cell responses detected by ICS or ELISPOT in C57BL/6 (WT) or β2i/MECL1$^{-/-}$β5i/LMP7$^{-/-}$ mice (KO) mice that were tattooed or infected, were tested for significance using Students T test. The variance homogeneity was tested using Levene's test. A Two-Way ANOVA, corrected for multiple comparisons using Tukey's correction was used to test for differ-