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Short communication

Influence of adenovirus and MVA vaccines on the breadth and hierarchy of T cell responses

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ABSTRACT

Viral-vectored vaccines are in clinical development for several infectious diseases where T-cell responses can mediate protection, and responses to sub-dominant epitopes is needed. Little is known about the influence of MVA or adenoviral vectors on the hierarchy of the dominant and sub-dominant T-cell epitopes. We investigated this aspect in mice using a malaria immunogen. Our results demonstrate that the T-cell hierarchy is influenced by the timing of analysis, rather than by the vector after a single immunization, with hierarchy changing over time. Repeated homologous immunization reduced the breadth of responses, while heterologous prime-boost induced the strongest response to the dominant epitope, albeit with only modest response to the sub-dominant epitopes.

1. Introduction

Leading vaccine strategies aiming at inducing strong cellular immunity in humans use recombinant adenovirus (Ad) and Modified Vaccinia Ankara (MVA) [1,2]. These vectors have been assessed since the late 90s for their capacity to induce different frequency, phenotype, function and localization of T- and B-cell responses [1,3–5]. A consensus emerged that priming with Ad followed by boosting with MVA was optimal to maintain strong and long-lived CD8+ T-cell responses [5,6], and thus many comparisons have been performed in the context of various heterologous prime – boost regimens [7–9], with few exceptions comparing them individually [3,10,11]. In the latter studies, T-cell responses were measured with tetramers [3], dominant epitopes [10], or peptide pools [11], but none investigated the dominance pattern of T-cell responses induced by each vaccine vector. However, it has been suggested that the immunogenicity of the vector is likely to have a strong influence on the transgenic antigen [12]. The objective of this study was to identify the influence of Ad and MVA vaccine vectors on the hierarchy and breadth of the T-cell epitope responses to the vaccine immunogen, and to specifically address the following four questions: (1) Is the hierarchy of T cell responses influenced by the vector? (2) Does the hierarchy of T cell responses change over time after a single immunization? (3) Do homologous or heterologous immunizations affect the T-cell hierarchy? (4) Is the hierarchy influenced by the timing between prime and boost?

2. Material and methods

2.1. Vectored vaccines

Human serotype 5 Ad and MVA vectors expressing the transgene ME.TRAP have been described previously [13]. The insert ME.TRAP encodes the *Plasmodium falciparum* TRAP and the immunodominant BALB/c H-2Kd epitope pb9 (CS252–260, SYIPSAEKI) from the *P. berghei* circumsporozoite protein.

2.2. Mice and immunizations

Animal experiments were performed in accordance with the U. K. Animals (Scientific Procedures) Act, 1986 and associated guidelines and EU Directive 2010/63/EU for animal experiments. Procedures were approved by the University of Oxford Animal Care and Ethical Review Committee. Groups of six 6 week-old female BALB/c mice (Harlan, UK) were injected intradermally in the ear pinna with 10^6 pfu for MVA or 5×10^9 vp for Ad, under short anesthesia using isoflurane. Ad + MVA vector mixtures (further referred as 'Mix') consisted of a single preparation containing both vectors at the same concentrations as each vector alone. All vaccines were injected in a final volume of 50 µl of phosphate-buffered saline.

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2.3. Ex vivo IFN-γ ELISPOT and peptide targets

Peripheral mononuclear cells (PBMCs) were stimulated with peptides at a final concentration of 1 μg/ml. ELISPOT was performed as previously described [13]. Results were expressed as the average spot forming units (SFU) per million cells. Peptides included the immunodominant Pb9, Ad hexon-specific H-2Kd-restricted epitope hex486–494 KYSPSNVKIA (Kia), Ad DNA-binding protein(dbp)-specific Ld-restricted epitope dbp413–421 LPKLTPFALA (Ala), MVA epitopes previously described E3, F2G [14] and SI9 [15]. Subdominant epitopes were identified in TRAP using 20mer peptides overlapping by 10 and covering TRAP aminoacid (aa) 101–310 from strain T9/96 [16]. This region was previously reported to elicit IFN-γ T-cell responses in BALB/c mice as opposed to peptides covering aa 1–110 and 301–564 (A. Spencer, personal communication and data not shown). Within this region, only epitopes 11 (IRLHSDASKNKEKALIIIRS), 12 (KEKALIIIRSLLSTNLPYGR), 17 (TDGIPDSIKDSLKESRKLSD), 20 (GQGINVAFNRFLVGCHPSDG), 22 (KCNLYADSAWENVKNVIGPF), 25 (TASCGVWDEWSPCSVTCG KG) and 30 (EPLDVPDEPEDDQPRPRGDN) induced detectable responses in at least 1 BALB/c mouse and are represented in the results.

3. Results and discussion

3.1. Influence of the vector and time on T-cell epitope hierarchy after a single injection

After a single injection with either Ad or MVA, or a mixture of both, T-cell responses were measured in blood at the peak of response, at week 1 for MVA and week 2 post-immunization for Ad [10], and after the contraction phase (week 10) (Fig. 1). Results are presented on a two-segment scale so that both the dominant (10,000–50,000 SFU/million cells) and subdominant (0–10,000 SFU/million cells) responses can be observed for all groups. One week after immunization, Ad induced responses only to the dominant epitope from the transgene, pb9, while responses induced by MVA were lower but broader, targeting peptides 22, 12 and 11 in addition (Fig. 1, top panels). Responses to the vector itself also differed: MVA epitope E3 was as strong as Pb9 in the MVA-immunized group, while the Ad hexon Kia was subdominant in the Ad group. Interestingly, the mixed modality combined the advantages of both vectors: a strong Pb9 response, detectable responses to peptides 22, 11 and 12 and low responses to the MVA vector epitopes. A week later (week 2), responses to the subdominant epitopes in the transgene were detected in the Ad-immunized group (peptides 22 > 20 > 12 > 17 > 25), with higher responses but similar hierarchy as compared with MVA immunization (Fig. 1, middle panels, responses to peptides ranked 22 > 20 > 12 > 17 > 25 in the MVA-immunized group). Of note, the response to the Ad dbp epitope Ala only appeared at week 2 and became dominant over the hexon epitope Kia, reflecting the late appearance of dbp being expressed from the vector while the hexon is present on the vaccine composition [17]. Responses had contracted after 10 weeks from injection. In the MVA-vaccinated animals, viral vector epitope E3 dominated and only the transgene pb9 epitope induced a detectable response (Fig. 1, bottom panels). In contrast, the response remained dominated by Pb9 in the Ad-vaccinated animals, but the subdominant hierarchy was modified, with peptides 20 and 11 still inducing detectable responses, while the response to peptide 22 was lost.

These results suggest that the hierarchy and breadth of T-cell epitope dominance in the transgene is not influenced by the vector, but rather by the timing of analysis after immunization, and the hierarchy changes overtime, which likely reflects affinity maturation and T cell proliferation [18].

3.2. Homologous boosts influence the hierarchy of T-cell responses

To establish whether the T-cell hierarchy is modified after homologous boosting, the same vector was administered after 10 weeks. Responses were measured 1 and 2 weeks post second

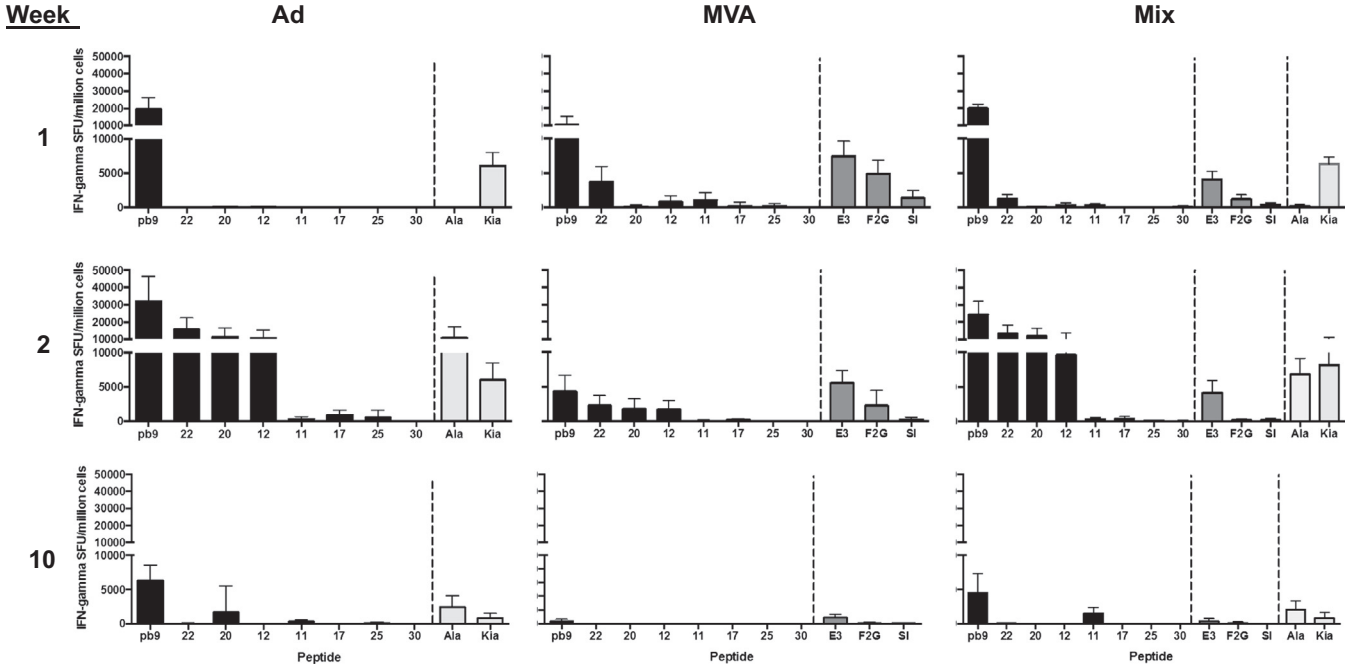

Fig. 1. T-cell responses after a single injection. Groups of mice were immunized at day 0 with Ad-METRAP, or MVA-METRAP or a mix of both (Mix), as indicated on the top. IFN-γ responses were measured in individual mice 1, 2 and 10 weeks post a single injection (as indicated on the left) by ELISPOT. Results are presented as the mean and SD for each group and each peptide.