Vaccine 27 (2009) 870–881

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Mucosal immunization in macaques upregulates the innate APOBEC 3G anti-viral factor in CD4+ memory T cells

Yufei Wang[a], Lesley A. Bergmeier[a], Richard Stebbings[b], Thomas Seidl[a], Trevor Whittall[a], Mahavir Singh[c], Neil Berry[b], Neil Almond[b], Thomas Lehner[a,*]

[a] Kings College London at Guy's Hospital, Mucosal Immunology Unit, Guy's Tower Floor 28, London SE1 9RT, England, United Kingdom
[b] National Institute for Biological Standards Control (NIBSC), Potters Bar, Herts EN6 3QG, England, United Kingdom
[c] Lionex GmbH and Helmholtz Center for Infection Research, 38124 Braunschweig, Germany

ARTICLE INFO

Article history:
Received 10 September 2008
Received in revised form
20 November 2008
Accepted 22 November 2008
Available online 11 December 2008

Keywords:
Mucosal immunization
APOBEC 3G
CD4+ memory T cells

ABSTRACT

APOBEC3G is an innate intracellular anti-viral factor which deaminates retroviral cytidine to uridine. *In vivo* studies of APOBEC3G (A3G) were carried out in rhesus macaques, following mucosal immunization with SIV antigens and CCR5 peptides, linked to the 70 kDa heat shock protein. A progressive increase in A3G mRNA was elicited in PBMC after each immunization ($p < 0.0002$ to $p \leq 0.02$), which was maintained for at least 17 weeks. Analysis of memory T cells showed a significant increase in A3G mRNA and protein in $CD4^+CCR5^+$ memory T cells in circulating ($p = 0.0001$), splenic ($p = 0.0001$), iliac lymph nodes ($p = 0.002$) and rectal ($p = 0.01$) cells of the immunized compared with unimmunized macaques. Mucosal challenge with SIVmac 251 showed a significant increase in A3G mRNA in the $CD4^+CCR5^+$ circulating cells ($p < 0.01$) and the draining iliac lymph node cells ($p < 0.05$) in the immunized uninfected macaques, consistent with a protective effect exerted by A3G. The results suggest that mucosal immunization in a non-human primate can induce features of a memory response to an innate anti-viral factor in $CCR5^+CD4^+$ memory and $CD4^+CD95^+CCR7^-$ effector memory T cells.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Innate immunity is a complex system of cellular and soluble factors directed against microorganisms and foreign molecules, which act independently of an immunological memory developed by prior encounter with an agent. The lack of an immunological memory, however has recently been challenged by the demonstration that NK cells can induce antigen-specific skin hypersensitivity reaction in the absence of T cells and that priming resulted in memory mediated by NK cells [1]. Furthermore, innate immunity via pattern recognition receptors, such as Toll-like receptors expressed on DC, activates antigen specific T and B cells and modulates the quantity and quality of T and B cell memory [2–5]. Immunological memory has been characterized by a rapid increase and prolonged specific immune response to an antigen. Memory T cells can be characterized by (a) phenotypic expression, (b) cytokines profile, (c) development of memory cell pathway and (d) anatomical site. An emerging paradigm in the prevention of HIV-1 infection is the development of a rapid innate immune response to the virus, in view of the infection and destruction of $CD4^+CCR5^+$ memory T cells within 2–3 weeks of HIV-1 transmission, mostly in the mucosally associated lymphoid tissue [6–9]. We attempted to focus attention on some of the criteria noted above, evaluating A3G expression within $CD4^+$ memory T cells in mucosally immunized and challenged non-human primates.

The first objective of this study was to find out if APOBEC3G (A3G) expression is elicited in rhesus macaques immunized by the mucosal route with SIV antigens and or CCR5 peptides linked to the HSP70 carrier. The second aim was to explore the possibility that immunization induces in $CD4^+$ T cells a memory-like function for A3G. The third objective was to find out if $CD4^+$ T cell innate and adaptive immunity are linked as exemplified by A3G expression and precursor frequency, respectively. The fourth objective was to evaluate the effect of A3G expression on the *in vivo* infectivity of $CD4^+$ T cells in the SIV challenged macaques. We have found progressive upregulation of A3G mRNA after each of three immunizations. Significant levels of A3G mRNA were maintained up to 47 weeks, which raised the possibility of the development of a memory-like response. This was supported by a significantly greater increase in A3G mRNA expression in the $CD4^+CCR5^+$ and $CD95^+$ memory T cells, and A3G protein in $CD4^+CD95^+$ $CCR7^-$ effector memory T cells. The A3G mRNA expression was significantly correlated with the precursor frequency of T cell proliferative response to SIVgp120 and CCR5 peptide. Furthermore, a significant increase in A3G mRNA was found in $CD4^+CCR5^+$ memory and in A3G

* Corresponding author. Tel.: +44 207 188 3072 fax: +44 207 188 4375.
E-mail address: thomas.lehner@kcl.ac.uk (T. Lehner).

0264-410X/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2008.11.084

protein in $CD4^+CD95^+CCR7^-$ effector memory T cells in immunized uninfected macaques.

2. Materials and methods

2.1. Preparation of the vaccine antigens and peptides

HSP70 derived from *Mycobacterium tuberculosis* was prepared in *Escherichia coli* as described previously [10]. It was purified by Q-sepharose followed by ATP affinity chromatography. The Q-Sepharose chromatography was repeated to remove endotoxin, which was tested by the Limulus amebocyte lysate assay and showed 1.2 pg of endotoxin per 1 μg HSP70 protein. The HSP70 preparation migrated as a doublet with molecular mass of approximately 70 kDa, with a minor component at approximately 35 kDa.

Recombinant SIVmac 251 gp120 was expressed in Baculovirus-infected cells and prepared by Oxford Expression Technologies. Supernatant (20 L) containing SIVgp120 was purified using monoclonal antibody (KK8) immobilised onto cyanogen bromide activated sepharose 4B beads (GE Healthcare, UK). The protein was eluted with 6 M guanidine and dialysed against sterile phosphate buffer. The purity of the protein was confirmed by SDS-PAGE and Western blotting. Recombinant SIVmac251 p27 was prepared by pGEX-3X as a glutathione S-transferase fusion protein from *E. coli*. The fusion protein has a molecular weight of about 56 kDa composed of SIVp27, molecular weight 27 kDa and the fusion partner gst at about 28 kDa. The protein was extracted from the bacterial cells by sonication and purified using gst affinity chromatography.

CCR5 peptides derived from the sequences of the N-terminal, loop 1 and loop 2 were synthesized to purity greater than 85%, as determined by HPLC and purchased from Bachem (Switzerland). The sequences of the peptides are shown below.

N-terminal (aa1-20): Met–Asp–Tyr–Gln–Val–Ser–Ser–Pro–ILe–Tyr Asp–ILe–Asp–Tyr–Tyr–Thr–Ser–Glu–Pro–Cys
Loop 1 (aa 89–102): His–Tyr–Ala–Ala–Ala–Gln–Trp–Asp–Phe–Gly –Asn–Thr–Met–Cys–Gln
Loop 2 (aa 178–197): Cys–Ser–Ser–His–Phe–Pro–Tyr–Ser–Gln–Tyr –Gln–Phe–Trp–Lys–Asn–Phe–Gln–Thr–Leu–Lys

2.2. Conjugation of HSP70 to the antigens and peptides

The HSP70 was conjugated to SIV gp120, SIV p27, N terminal and 2nd loop of CCR5 by means of the SPDP reagent which is less likely to alter the immunogenicity of the vaccine components than glutaraldehyde [11]. First loop was non-covalently linked to HSP70, as this peptide binds directly to the peptide binding groove, demonstrated both by surface plasmon resonance and by immunization in mice. At each stage of the SPDP substitution and conjugation the HSP70/protein or peptide complex was subjected to SDS-PAGE and Western blot analysis. Under non-reducing conditions high molecular weight complexes were observed which stained for both HSP70 and the conjugated protein, indicating successful conjugation. Under reducing conditions the complexes were broken down into their constituent molecules. HSP70-SIV p27 complex contained a small amount of free SIV p27.

2.3. Antibodies

The following mAb were used for immunofluorescence studies with the macaque cells: mAb to human CD95 (clone: DX, allophy-

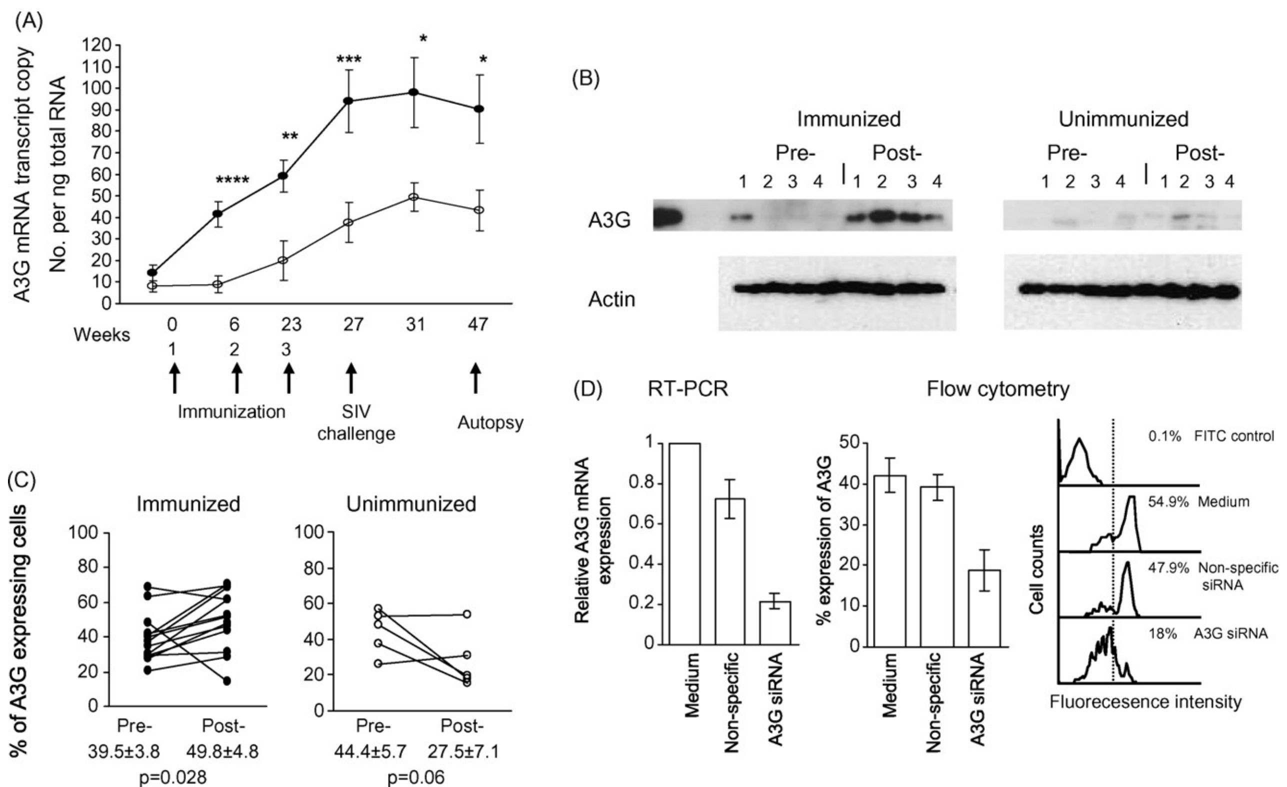

Fig. 1. (A) Sequential A3G mRNA expression in PBMC before and after (×3) immunization (●) n = 15 and unimmunized control (○) n = 5 rhesus macaques, which were challenged with SIVmac251 (at week 27, 4 weeks after the 3rd immunization). Blood was collected before each of the 3 immunizations and SIV challenge. The results are presented as mean ±sem; $\leq 0.02^{*}$, $<0.01^{**}$, $<0.005^{***}$, $<0.0002^{****}$. (B) Western blots before and after the 3rd immunization (week 27) with lysates of PBMC from 4 macaques. (C) Intracellular expression of A3G proteins in $CD4^+$ T cells detected by flow cytometry before and after 3rd immunization (week 27). (D) A3G mRNA and protein knock down with A3G specific and non-specific siRNA of $CD4^+$ T cells from 4 animals and representative flow cytometry.

Download English Version:

https://daneshyari.com/en/article/10970330

Download Persian Version:

https://daneshyari.com/article/10970330

Daneshyari.com