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ScienceDirect
Virology 361 (2007) 274–282
VIROLOGY
www.elsevier.com/locate/yviro

Efficient vagina-to-lower respiratory tract immune trafficking in a murine model of influenza A virus infection

Bruno Garulli [a,b], Monica Meola [a], Maria Giuseppina Stillitano [a], Yoshihiro Kawaoka [c,d,e], Maria Rita Castrucci [a,*]

[a] Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[b] Department of Cellular and Developmental Biology, University of Rome "La Sapienza", 00185 Rome, Italy
[c] Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, WI 53706, USA
[d] Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
[e] CREST, Japan Science and Technology Agency, Saitama 332-0012, Japan

Received 21 July 2006; returned to author for revision 23 August 2006; accepted 1 December 2006
Available online 11 January 2007

Abstract

Effective vaccination strategies for infectious diseases take into account the induction, long-term maintenance and recall of memory T-cell populations. To understand the immunological cross-talk within the mucosal compartments, we compared intranasal to vaginal immunization and demonstrated that vaginal infection of BALB/c mice with influenza A virus provides protective mucosal immunity against both homosubtypic and heterosubtypic virus challenge in the respiratory tract. We found that, prior to the viral challenge, in vaginally primed mice, antigen-specific CD8+ T cells were not detected in the lung airways and levels of serum antibodies were lower than those observed in intranasally immunized mice. However, following pulmonary challenge, NP147-specific CD8+ T cells were recruited and amplified in vaginally primed mice to the same extent as those in intranasally primed mice. Thus, the long-term memory immune response elicited by vaginal immunization with influenza virus is efficiently recalled and offers reasonable protection against infection in the respiratory tract.

Keywords: Influenza A virus; Heterosubtypic immunity; Memory T-cell responses; Vaginal immunization

Introduction

Establishing both strong mucosal and systemic immune responses is important for the prevention of most mucosa-contracted infections. Indeed, the common mucosal immune system allows immunological cross-talk within different mucosal effector sites, increasing the options for vaccination (Bienestock et al., 1992; McDermott and Bienenstock, 1979; Staats et al., 1994). Intranasal immunization is known to efficiently stimulate humoral and cell-mediated immune responses at both the systemic and mucosal levels. Furthermore, nasal immunization is an effective route of cross-immunization within the integrated mucosal immune system since it induces long-term specific immunity in the genital tract (Dupuy et al., 1999; Ferko et al., 2001; Gallichan and Rosenthal, 1996, 1998; Gherardi et al., 2003; Klavinskis et al., 1999; Muster et al., 1995). In contrast, limited information is available concerning immune trafficking to the respiratory tract upon vaginal immunization.

We recently showed that progesterone-treated BALB/c mice support the replication of influenza A viruses in vaginal tissues (Garulli et al., 2004). A single inoculation of an influenza virus via the vaginal route induced specific humoral and cellular immune responses both in the spleen and in regional lymph nodes. Moreover, upon vaginal immunization, mice generated long-term virus-specific memory cytotoxic T lymphocytes (CTLs) in the spleen and in the mucosal lymph nodes that drain the genital tract, as detected by a rapid recall of effector CTLs on re-exposure to the antigen. However, the extent to which such memory responses afford protection from challenge with pathogens remains unknown.

* Corresponding author. Fax: +39 06 49902082.
E-mail addresses: bruno.garulli@uniroma1.it (B. Garulli), kawaokay@svm.vetmed.wisc.edu (Y. Kawaoka), maria.castrucci@iss.it (M.R. Castrucci).

0042-6822/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2006.12.001

We, therefore, compared the efficacy of vaginal versus intranasal immunization to protect against pulmonary infection of homosubtypic or heterosubtypic influenza A virus. Our data show that vaginal immunization with influenza virus effectively primes long-term, sustained protective immune responses in the pulmonary organs, albeit to a lesser extent than intranasal priming.

Results

Induction of local and systemic memory CTLs cross-reactive with viruses of different subtypes following vaginal infection with influenza A virus

In our previous study (Garulli et al., 2004), we showed that vaginal immunization with a recombinant influenza virus bearing an HIV-1 T-cell epitope (Flu/P18IIIB) induced a vigorous immune response at both mucosal and systemic levels against influenza viral and the HIV-1 epitopes. Here, we asked whether the subtype cross-reactive CD8+ T-cell responses can be induced in spleen and iliac lymph nodes (ILN) by vaginal infection of mice with influenza A viruses. Groups of BALB/c mice were first vaginally primed by infection with 3×10^5 PFU of X31 virus (H3N2). Six months after priming, these mice, and age-matched control mice, were vaginally infected again with 10^6 PFU of the serologically distinct WSN virus (H1N1) that shares the NP147 immunodominant epitope (H-2K^d) with the X31 virus. Mice were sacrificed either 3 days or 5 days postinfection and their regional lymph nodes and spleens assayed for NP-specific CTL activity. To achieve this, ILN-derived lymphocytes, cultured *in vitro* for 3 days without exogenous antigen, and splenocytes that had undergone antigen-specific stimulation for 6 days were examined for their ability to lyse ^{51}Cr-labeled P815 target cells pulsed with the peptide NP147. One characteristic example of ^{51}Cr release data at an effector-to-target ratio of 50 to 1 is shown in Fig. 1. In the control (unprimed) mice, the CTL response to WSN virus in ILN-derived cells (Fig. 1A) was detectable on day 5 but not day 3 postinfection and was barely detectable in splenocytes at the day 5 time point (Fig. 1B). By contrast, the CTL response of X31-primed mice measured after WSN challenge was substantially higher than that of the control, unprimed mice in both the ILN-derived cells and splenocytes, on day 3 and day 5 postinfection. The effector cells that lysed peptide-pulsed MHC-compatible target cells exhibited only background levels of lytic activity against unpulsed P815 target cells or allospecific EL4 cells (data not shown).

Thus, vaginal immunization with influenza virus resulted in efficient induction and long-term maintenance of virus cross-reactive NP-specific CTLs in local draining lymph nodes and the spleen.

Long-lived memory CD8+ T-cell responses to heterosubtypic influenza A virus in the draining lymph nodes of the respiratory tract of vaginally primed mice following intranasal challenge

To understand the cross-talk between the two different mucosal compartments, we next examined immune trafficking to the respiratory organs in mice vaginally immunized with influenza virus. To this end, we compared the induction and maintenance of long-term memory NP147-specific CD8+ T cells in mice primed by the vaginal route relative to that in mice primed by the intranasal route. Freshly isolated NP147-specific CD8+ T lymphocytes in the mediastinal lymph nodes (MLN) that drain the respiratory tract were assayed for reactivity with an NP147/K^d tetramer in groups of naive and immune (intranasally or vaginally primed) mice following intranasal challenge with WSN virus (Flynn et al., 1998). We found that,

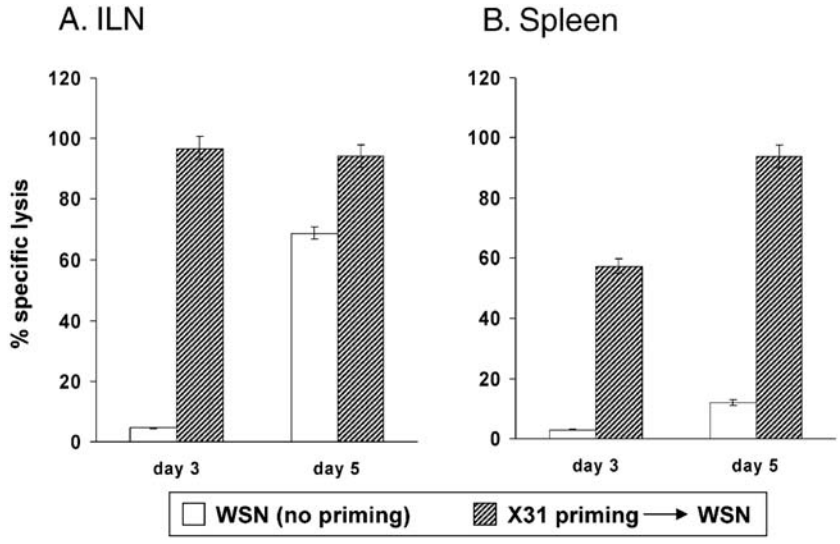

Fig. 1. Long-lived specific memory CTL activity in mice vaginally infected with X31 virus. Groups of five BALB/c mice were vaginally infected with 3×10^5 PFU of X31 virus and 6 months later infected with 10^6 PFU of WSN virus. ILN (A) and spleens (B) were removed 3 or 5 days after the vaginal infection with WSN virus. The ILN were cultured without antigen stimulation for 3 days before being tested in a CTL assay. Spleen cells were stimulated for 6 days *in vitro* with irradiated NP147 peptide-loaded stimulators before being examined for CTL activity. CTLs were assayed with NP147 peptide-pulsed P815 cells as targets. Mean lysis values (±SD) measured at an effector-to-target ratio of 50:1 are shown.