REVIEW 10.1111/j.1469-0691.2012.03995.x

B cell and T cell immunity in the female genital tract: Potential of distinct mucosal routes of vaccination and role of tissue-associated dendritic cells and natural killer cells

F. Anjuère[1,2], S. Bekri[1,2], F. Bihl[1,2], V.M. Braud[1,2], N. Cuburu[3], C. Czerkinsky[4], C. Hervouet[5] and C. Luci[1,2]

1) *CNRS, UMR7275 CNRS/UNS, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne,* 2) *University of Nice-Sophia Antipolis, Nice, France,* 3) *Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD, USA,* 4) *International Vaccine Institute, Seoul, Korea and* 5) *Department of Immunobiology, King's College London, London, UK*

Abstract

The female genital mucosa constitutes the major port of entry of sexually transmitted infections. Most genital microbial pathogens represent an enormous challenge for developing vaccines that can induce genital immunity that will prevent their transmission. It is now established that long-lasting protective immunity at mucosal surfaces has to involve local B-cell and T-cell effectors as well as local memory cells. Mucosal immunization constitutes an attractive way to generate systemic and genital B-cell and T-cell immune responses that can control early infection by sexually transmitted pathogens. Nevertheless, no mucosal vaccines against sexually transmitted infections are approved for human use. The mucosa-associated immune system is highly compartmentalized and the selection of any particular route or combinations of routes of immunization is critical when defining vaccine strategies against genital infections. Furthermore, mucosal surfaces are complex immunocompetent tissues that comprise antigen-presenting cells and also innate immune effectors and non-immune cells that can act as 'natural adjuvants' or negative immune modulators. The functions of these cells have to be taken into account when designing tissue-specific antigen-delivery systems and adjuvants. Here, we will discuss data that compare different mucosal routes of immunization to generate B-cell and T-cell responses in the genital tract, with a special emphasis on the newly described sublingual route of immunization. We will also summarize data on the understanding of the effector and induction mechanisms of genital immunity that may influence the development of vaccine strategies against genital infections.

Keywords: Dendritic cells, genital immunity, mucosal vaccines, natural killer cells, tissue-associated memory T cells
Original Submission: 7 May 2012; **Accepted:** 13 July 2012
Editor: M. Paul
Clin Microbiol Infect 2012; **18** (Suppl. 5): 117–122

Corresponding author: F. Anjuère, Centre National de la Recherche Scientifique, CNRS/UNSA UMR 7275, Institut de Pharmacologie Moléculaire et Cellulaire, 660 Route des Lucioles, 06560 Valbonne, France
E-mail: anjuere@unice.fr

Introduction

The female genital tract consists of the upper reproductive tract (uterus and cervix) and the lower reproductive tract (vagina). It constitutes the major port of entry of several pathogens such as human immunodeficiency virus (HIV), herpes simplex virus (HSV), human papillomavirus, *Chlamydia* and *Neisseria gonorrhoeae*. These sexually transmitted infections (STI) represent a major public health problem in both industrialized and developing countries. Hence, development of vaccines that can induce a strong genital immunity preventing STI transmission is crucial but challenging. Indeed, despite numerous efforts, the sole human vaccines licensed to prevent an STI until now are the human papillomavirus vaccines Gardasil® and Cervarix®. These prophylactic vaccines that are given by intramuscular injection are believed to confer protection by inducing genital neutralizing antibodies derived

from the circulation [1–3]. For other pathogens, in particular HIV and HSV-2, a protective immunity has to elicit strong B-cell and T-cell responses both at the site of infection and in the periphery to block systemic spreading [4–6]. Ideally, a vaccine against genital infection should induce genital and systemic immune responses involving effector B-cell, CD4 and CD8 T-cell responses as well as local B and T memory cells.

The mucosa-associated immune system is highly compartmentalized. Mucosal immunization, unlike parenteral immunization, is able to favour the generation of secretory antibodies (IgA) and CD4 and CD8 T cells both at the site of mucosal immunization and in distant mucosae under certain conditions [7,8]. Interestingly, Gallichan and Rosenthal demonstrated that mucosal immunization generates mucosal long-term CD8 T cells, in contrast to parenteral immunization, and that these tissue-associated memory T cells are critical for antiviral protection against mucosal challenge [9]. Hence, characterizing the phenotype of long-term tissue-associated effector immune cells in the genital mucosa and identifying the cellular and molecular mechanisms that induce their generation and their specific homing in the genital tract are of particular interest for development of vaccines against STI. Moreover, the generation of efficient mucosal and systemic immune responses following mucosal immunization requires the use of appropriate adjuvants and antigen-delivery systems [10–12]. These vaccine components will influence the quality and the nature of the immunity needed at a desired site of the body.

Therefore, the design of vaccines against STI must take into account the routes of immunization as well as the nature of the components of the vaccines themselves. A major question to be answered is where and how a vaccine against an STI has to be delivered to generate a robust and tissue-specific long-lasting immunity in the female genital tract. In this review, we will summarize data that compare different mucosal routes of immunization to generate B-cell and T-cell responses in the genital tract, with a special emphasis on the newly described sublingual route of immunization. We will also discuss data available on effector and induction mechanisms of mucosal immunity that may influence the development of vaccine strategies against genital infections.

Routes of Immunization and B-cell and T-cell Immunity in the Female Genital Tract

Comparison of mucosal and parenteral routes of immunization

Numerous studies have compared the potential of different mucosal routes of immunization to generate IgG and IgA antibody responses in the human and mouse cervico-vaginal mucosae after topical application of non-replicating vaccines as reviewed elsewhere [11]. Vaginal immunization was shown to induce IgA and IgG antibody-secreting cells in vagina and uterus as well as cervico-vaginal and seric antibodies [13–15]. Furthermore, nasal immunization gave rise to IgA and IgG antibody responses in human cervico-vaginal washes with intensities comparable to those seen following vaginal immunization [14]. Several studies comparing different routes of immunization with replicating or non-replicating model antigens in mice have also documented that nasal immunization can induce IgA and IgG antibodies as well as effector T cells in the genital tract [15–17]. The potential of the sublingual route for delivering vaccines has gained interest because it favours the induction of broadly disseminated mucosal and systemic immune responses [18,19]. Notably, sublingual immunization was as potent as vaginal immunization for the induction of IgA and IgG antibodies and cytotoxic T cells in the female genital tract, in contrast to parenteral immunization [15,20]. Furthermore, sublingual immunization with human papillomavirus-like particles evoked virus-neutralizing antibody responses in both serum and cervico-vaginal washes, and provided protection against genital challenge with human papillomavirus pseudovirions [15]. In addition, sublingual immunization of female macaques with an HIV subunit vaccine induced IgG and IgA antibody responses in the genital tract (F. Anjuère, unpublished results). It should also be noted that the sublingual route of immunization reduces the risk that antigens and adjuvants are redirected to the olfactory bulb epithelium, as observed after intranasal vaccination. Altogether, these studies suggest that this novel route of immunization may constitute an interesting alternative route to intranasal immunization for human vaccination against urogenital infections.

Combination of routes of immunization

Recent studies indicated that a prime-boost strategy comprising a mucosal prime followed by an intramuscular boost immunization was beneficial to generate protective immunity either in macaques against a challenge by simian HIV [21] or in mice against *Chlamydia* genital shedding [22]. In both studies, the mucosal immunization (intranasal or sublingual) was able to induce a protective immunity in combination with an intramuscular immunization whereas parenteral immunization alone was not. Another recent study in mice highlighted that a local vaginal boost immunization after a nasal prime is even superior to intranasal boost to induce protection against *Chlamydia* infection [23]. Furthermore, vaginal prime/boost strategy using two recombinant vectors was more efficient than other routes of immunization (systemic and mucosal) to

induce a protective immunity against a genital challenge with a recombinant vaccinia virus [24]. In these studies, local immunization was shown to favour the amplification and maintenance of long-lived tissue-resident CD4 and CD8 T cells that play a central role in protection against most genital pathogens.

Generation of memory responses in the female genital tract

Recent studies showed that cutaneous and intravaginal viral infections in mice induce the generation of high-affinity tissue-resident CD8 memory T cells that are critical for the control of epithelial pathogens re-encountered at their port of entry [25,26]. Interestingly, Gallichan and Rosenthal demonstrated that mucosal immunization generates mucosal long-term CD8 T cells in contrast to parenteral immunization and, that these T cells were critical for antiviral protection against a mucosal challenge [9]. Hence, the generation of tissue-resident memory T cells must be a goal of vaccination as reviewed elsewhere [27]. A question that arises is whether replication-defective vaccines can be as efficient as pathogens themselves or as live vaccines to generate these cells? As a non-replicating *Chlamydia* vaccine was able to generate long-term protective CD4 T cells in the genital tract, this suggests that it is feasible [23]. The next steps toward the development of vaccines generating memory T cells in the genital tract will be to further characterize these cells and to identify the local factors that favour their vaginal expansion and maintenance.

Adjuvants

The adjuvants, the antigen-delivery systems and the vaccine formulations employed will have profound influences on the intensity and the nature of mucosal immune responses generated. Potent immunomodulatory agents, including toxin-based adjuvants, Toll-like receptor mimetics and non-Toll-like receptor-targeting immunostimulators as well as delivery systems are under evaluation in animal models and may represent components of future vaccines as extensively detailed elsewhere [11,12,28,29]. Such immune modulators will be useful for the development of vaccines against genital infections if they can improve the development of vagina-associated memory B and T cells.

Homing of Effector Cells in the Female Genital Tract

The homing of lymphocytes to specific tissues is a key component of mucosal immunity and is under the control of chemokine receptors and integrins expressed by effector cells as well as addressins and chemokines expressed in tissues. It is already well known that the selective expression of the integrin $\alpha_4\beta_7$ and the chemokine receptor CCR9 by effector B and T cells determines their homing to gut-associated lymphoid tissue [30,31]. In contrast, the homing phenotype of B and T effector and memory cells to the female genital mucosa is partially understood. Recent data indicated that the chemokine receptor CCR10 plays a role in the migration of IgA-producing plasma cells to the genital tissue in response to the chemokine CCL28 by a mechanism that is dependent on oestrogens [15,32]. Both studies also demonstrated that the IgA-specific CCR10$^+$ plasma cells produced by sublingual and nasal immunizations preferentially migrate to the uterine mucosa [15,32]. Furthermore, the expression of the αE integrin (CD103) by cervical human gag-specific CD8 T cells [33] and by genital HSV-specific memory T cells [26] suggested that CD103 expression is important for the mucosal homing and for the maintenance of T cells in the female genital tract. A question that then arises is what are the cellular and molecular mechanisms involved in the homing and the retention of effector and memory cells in the female genital tract.

Role of Dendritic Cells in the Generation of Genital Effectors

Dendritic cells (DCs) represent professional antigen-presenting cells that have dual roles: the initiation of productive adaptive immunity including the generation of anti-tumour cytotoxic T cells and the induction of immunoregulation through the differentiation of regulatory T-cell subsets. The vaginal mucosa comprises distinct subsets of DCs including intra-epithelial DCs expressing or not the langerin marker, as well as submucosal DCs [34,35]. The presentation of non-replicating antigens or pathogens following vaginal immunization was shown to mainly involve DCs able to locally take up and process the antigen, and to carry it to draining lymph nodes to prime naive CD4 and CD8 T cells [6]. We and others have demonstrated that the mucosal administration of a protein antigen with cholera toxin used as adjuvant induces the transient recruitment of immunostimulatory DCs at the site of immunization, which triggers the generation of systemic and mucosal CD8$^+$ effector T cells [36–38]. Interestingly, a subset of CD11b$^+$ DCs recruited in the vagina after local stimulation played a crucial role in the generation of vaginal cytotoxic CD8 T-cell responses whereas langerin-expressing DCs dampened these responses [35]. Vaginal submucosal CD11b$^+$ DCs were also involved in the presentation of HSV-derived antigens to CD4 T cells following HSV-2 intravaginal infection [39]. These data indicate that submuco-

sal CD11b$^+$ DCs, but not Langerhans cells, may constitute a potential target for vaccines that aim to generate protective genital CD4 and CD8 T cells. This would require the identification of an appropriate surface marker restricted to this particular DC subset. Vaginal submucosal DCs share the ability to cross-present exogenous antigens with dermal XCR1$^+$ CD103$^+$ DCs, which indicates that, if expressed on mucosal CD11b$^+$ DCs, these markers might be useful to develop specific DC-targeted vaccines [38,40]. Nevertheless, further studies are needed to identify markers restricted to submucosal CD11b$^+$ DCs. Furthermore, tissue-associated DCs were reported to produce factors that can influence the expression of mucosal homing markers by activated T and B cells. This has been well documented in the gastrointestinal tract, where a specific CD103$^+$ DC subset that metabolizes retinoic acid induced the expression of the integrin $\alpha_4\beta_7$ and the chemokine CCR9 on activated T cells and on antibody-secreting cells, so favouring their migration to gut-associated lymphoid tissue [30,41]. This discovery may have implications in the field of mucosal immunization because DCs from non-intestinal epithelial sites do not produce retinoic acid. Interestingly, it was recently shown that adding retinoic acid to a vaccine preparation was sufficient to induce the migration of CD8 effector T cells to the vaginal mucosa and to protect mice against a genital challenge with HSV [42], indicating that retinoic acid might be a component of vaccines that aim to elicit genital immunity.

Consequently, as vaginal CD11b$^+$ DCs induce the homing of T-cell effectors to the vaginal mucosa, one can ask whether and how these DCs can imprint vaginal T cells and whether one can identify a surface marker selectively expressed by this DC subset to develop a DC-targeted vaccine against genital infections.

Mucosal Innate Effector Cells as Immune Modulators of Genital B-cell and T-cell Immunity

Different subsets of innate cells are present or recruited to mucosae following inflammatory processes and infections where they play a major role in innate defense. Natural killer (NK) cells are cytotoxic lymphocytes of the innate immune system [43,44], and are potent immune modulators of adaptive immunity during infections either by exerting their cytolytic activity on effector and central memory T lymphocytes [45–48], or by killing antigen-presenting DCs [49]. Furthermore, NK cells have been recently identified in sub-epithelial tissues (skin, intestine, tonsils) in humans and mice [50–52], but they remain to be fully characterized in the female genital tract. Nevertheless, the contribution of NK cells in protection against intravaginal HSV-2 infection was evaluated using animal models where the differentiation of NK cells is affected. These studies suggested that NK cells are crucial for the viral clearance even if the models used did not specifically deplete NK cells [53,54]. Further experiments are needed to evaluate the role of NK cells in vaginal antiviral immunity. Furthermore, a question that also arises is whether vaginal NK cells can modulate genital T-cell and B-cell immunity and how? Recently, interleukin-6-producing NK cells characterized in the lymph nodes draining the nasal mucosa were shown to potentiate the induction of mucosal antibodies after nasal immunization, showing that mucosa-derived NK cells can contribute to the generation of mucosal adaptive immunity [55].

Different subsets of NK T lymphocytes have been mainly studied in the skin. They have also been recently identified in the intestinal mucosa [56] but their presence and role in pluristratified mucosae remain to be established. Nevertheless, the nasal administration of the αGalCer molecule as mucosal adjuvant together with an HSV antigen was shown to increase the generation of HSV-specific effector T cells and to improve the protection of mice against a genital challenge by HSV-2 [57]. This study suggests that the activation of mucosal NKT cells by αGalCer ligand potentiates adaptive immune responses induced by mucosal immunization.

Further characterization of NK and NKT cells present in the vaginal mucosa and other mucosal sites of induction of genital immunity (nasal and sublingual mucosae) is needed to elucidate their functional properties during mucosal vaccination, as well as their impact on genital effector responses. A better understanding of the functions of these innate cells following mucosal immunization may help to optimize vaccines strategies against STI.

Conclusions

Recent data highlighted that mucosal immunization or combinations of mucosal and parenteral immunizations are crucial for the generation of protective immunity against different genital pathogens, probably because these vaccine strategies favour the expansion of long-lasting B-cell and T-cell responses in the genital tract. Future research should be dedicated to a better understanding of mechanisms of induction of genital B-cell and T-cell immunity. Particular attention should be paid to the role of tissue-associated dendritic cell subsets and to the emerging role of mucosa-associated innate immune cells in the generation of protective immunity, as well as to the mechanisms of homing and retention of cervico-vaginal memory cells which are critical for protection.

Transparency Declaration

Our work is supported by Centre National de la Recherche Scientifique (France), Institut National de la Santé et de la Recherche Médicale (France), EU-FP7 Euroneut41 project, the Agence Nationale de Recherche sur le SIDA (France), the SIDACTION (France), the Association de Recherche sur le Cancer (ARC) and the Fondation de l'Avenir (France).

Conflicts of interest

All the authors declare no conflict of interest.

References

1. Stanley MA. Epithelial cell responses to infection with human papillomavirus. *Clin Microbiol Rev* 2012; 25: 215–222.
2. Frazer IH, Lowy DR, Schiller JT. Prevention of cancer through immunization: prospects and challenges for the 21st century. *Eur J Immunol* 2007; 37 (Suppl 1): S148–S155.
3. Day PM, Kines RC, Thompson CD *et al. In vivo* mechanisms of vaccine-induced protection against HPV infection. *Cell Host Microbe* 2010; 8: 260–270.
4. Koup RA, Douek DC. Vaccine design for CD8 T lymphocyte responses. *Cold Spring Harb Perspect Med* 2011; 1: a007252.
5. Girard MP, Osmanov S, Assossou OM, Kieny MP. Human immunodeficiency virus (HIV) immunopathogenesis and vaccine development: a review. *Vaccine* 2011; 29: 6191–6218.
6. Iwasaki A. Antiviral immune responses in the genital tract: clues for vaccines. *Nat Rev Immunol* 2010; 10: 699–711.
7. Czerkinsky C, Anjuere F, McGhee JR *et al.* Mucosal immunity and tolerance: relevance to vaccine development. *Immunol Rev* 1999; 170: 197–222.
8. Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. *Nat Med* 2005; 4 (Suppl): S45–S53.
9. Gallichan WS, Rosenthal KL. Long-lived cytotoxic T lymphocyte memory in mucosal tissues after mucosal but not systemic immunization. *J Exp Med* 1996; 184: 1879–1890.
10. Anjuere F, Czerkinsky C. Mucosal immunity and vaccine development. *Med Sci (Paris)* 2007; 23: 371–378.
11. Czerkinsky C, Holmgren J. Topical immunization strategies. *Mucosal Immunol* 2010; 3: 545–555.
12. Harandi AM, Medaglini D. Mucosal adjuvants. *Curr HIV Res* 2010; 8: 330–335.
13. Kozlowski PA, Cu-Uvin S, Neutra MR, Flanigan TP. Comparison of the oral, rectal, and vaginal immunization routes for induction of antibodies in rectal and genital tract secretions of women. *Infect Immun* 1997; 65: 1387–1394.
14. Johansson EL, Rask C, Fredriksson M, Eriksson K, Czerkinsky C, Holmgren J. Antibodies and antibody-secreting cells in the female genital tract after vaginal or intranasal immunization with cholera toxin B subunit or conjugates. *Infect Immun* 1998; 66: 514–520.
15. Cuburu N, Kweon MN, Hervouet C *et al.* Sublingual immunization with nonreplicating antigens induces antibody-forming cells and cytotoxic T cells in the female genital tract mucosa and protects against genital papillomavirus infection. *J Immunol* 2009; 183: 7851–7859.
16. Rosenthal KL, Gallichan WS. Challenges for vaccination against sexually transmitted diseases: induction and long-term maintenance of mucosal immune responses in the female genital tract. *Semin Immunol* 1997; 9: 303–314.
17. Del Campo J, Lindqvist M, Cuello M *et al.* Intranasal immunization with a proteoliposome-derived cochleate containing recombinant GD protein confers protective immunity against genital herpes in mice. *Vaccine* 2010; 28: 1193–1200.
18. Czerkinsky C, Cuburu N, Kweon MN, Anjuere F, Holmgren J. Sublingual vaccination. *Hum Vaccin* 2011; 7: 110–114.
19. Kweon MN. Sublingual mucosa: a new vaccination route for systemic and mucosal immunity. *Cytokine* 2011; 54: 1–5.
20. Hervouet C, Luci C, Cuburu N *et al.* Sublingual immunization with an HIV subunit vaccine induces antibodies and cytotoxic T cells in the mouse female genital tract. *Vaccine* 2010; 28: 5582–5590.
21. Bomsel M, Tudor D, Drillet AS *et al.* Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. *Immunity* 2011; 34: 269–280.
22. Carmichael JR, Pal S, Tifrea D, de la Maza LM. Induction of protection against vaginal shedding and infertility by a recombinant chlamydia vaccine. *Vaccine* 2011; 29: 5276–5283.
23. Marks E, Tam MA, Lycke NY. The female lower genital tract is a privileged compartment with IL-10 producing dendritic cells and poor Th1 immunity following *Chlamydia trachomatis* infection. *PLoS Pathog* 2010; 6: e1001179.
24. Li Z, Zhang M, Zhou C, Zhao X, Iijima N, Frankel FR. Novel vaccination protocol with two live mucosal vectors elicits strong cell-mediated immunity in the vagina and protects against vaginal virus challenge. *J Immunol* 2008; 180: 2504–2513.
25. Jiang X, Clark RA, Liu L, Wagers AJ, Fuhlbrigge RC, Kupper TS. Skin infection generates non-migratory memory $CD8^+$ T(rm) cells providing global skin immunity. *Nature* 2012; 483: 227–231.
26. Tang VA, Rosenthal KL. Intravaginal infection with herpes simplex virus type-2 (HSV-2) generates a functional effector memory T cell population that persists in the murine genital tract. *J Reprod Immunol* 2010; 87: 39–44.
27. Kupper TS. Old and new: recent innovations in vaccine biology and skin T cells. *J Invest Dermatol* 2012; 132(3 Pt 2): 829–834.
28. Lawson LB, Norton EB, Clements JD. Defending the mucosa: adjuvant and carrier formulations for mucosal immunity. *Curr Opin Immunol* 2011; 23: 414–420.
29. Luci C, Anjuere F. IFN-λ and $BDCA3^+$/$CD8\alpha^+$ dendritic cells: towards the design of novel vaccine adjuvants? *Expert Rev Vaccines* 2011; 10: 159–161.
30. Mora JR, Bono MR, Manjunath N *et al.* Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* 2003; 424: 88–93.
31. Sigmundsdottir H, Butcher EC. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. *Nat Immunol* 2008; 9: 981–987.
32. Cha HR, Ko HJ, Kim ED *et al.* Mucosa-associated epithelial chemokine/CCL28 expression in the uterus attracts $CCR10^+$ IgA plasma cells following mucosal vaccination via estrogen control. *J Immunol* 2011; 187: 3044–3052.
33. Kiravu A, Gumbi P, Mkhize NN, Olivier A, Denny L, Passmore JA. Evaluation of CD103 ($\alpha_e\beta_7$) integrin expression by CD8 T cells in blood as a surrogate marker to predict cervical T cell responses in the female genital tract during HIV infection. *Clin Immunol* 2011; 141: 143–151.
34. Iijima N, Linehan MM, Saeland S, Iwasaki A. Vaginal epithelial dendritic cells renew from bone marrow precursors. *Proc Natl Acad Sci U S A* 2007; 104: 19061–19066.
35. Hervouet C, Luci C, Rol N *et al.* Langerhans cells prime IL-17-producing T cells and dampen genital cytotoxic responses following mucosal immunization. *J Immunol* 2010; 184: 4842–4851.

36. Le Borgne M, Etchart N, Goubier A *et al.* Dendritic cells rapidly recruited into epithelial tissues via CCR6/CCL20 are responsible for $CD8^+$ T cell crosspriming *in vivo*. *Immunity* 2006; 24: 191–201.
37. Anjuere F, Luci C, Lebens M *et al. In vivo* adjuvant-induced mobilization and maturation of gut dendritic cells after oral administration of cholera toxin. *J Immunol* 2004; 173: 5103–5111.
38. Luci C, Hervouet C, Rousseau D, Holmgren J, Czerkinsky C, Anjuere F. Dendritic cell-mediated induction of mucosal cytotoxic responses following intravaginal immunization with the nontoxic B subunit of cholera toxin. *J Immunol* 2006; 176: 2749–2757.
39. Lee HK, Zamora M, Linehan MM *et al.* Differential roles of migratory and resident DCs in T cell priming after mucosal or skin HSV-1 infection. *J Exp Med* 2009; 206: 359–370.
40. Crozat K, Tamoutounour S, Vu Manh TP *et al.* Cutting edge: expression of XCR1 defines mouse lymphoid-tissue resident and migratory dendritic cells of the $CD8\alpha^+$ type. *J Immunol* 2011; 187: 4411–4415.
41. Johansson-Lindbom B, Svensson M, Pabst O *et al.* Functional specialization of gut $CD103^+$ dendritic cells in the regulation of tissue-selective T cell homing. *J Exp Med* 2005; 202: 1063–1073.
42. Tan X, Sande JL, Pufnock JS, Blattman JN, Greenberg PD. Retinoic acid as a vaccine adjuvant enhances $CD8^+$ T cell response and mucosal protection from viral challenge. *J Virol* 2011; 85: 8316–8327.
43. Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural-killer cells and dendritic cells: "L'union fait la force". *Blood* 2005; 106: 2252–2258.
44. Luci C, Tomasello E. Natural killer cells: detectors of stress. *Int J Biochem Cell Biol* 2008; 40: 2335–2340.
45. Soderquest K, Walzer T, Zafirova B *et al.* Cutting edge: $CD8^+$ T cell priming in the absence of NK cells leads to enhanced memory responses. *J Immunol* 2011; 186: 3304–3308.
46. Waggoner SN, Cornberg M, Selin LK, Welsh RM. Natural killer cells act as rheostats modulating antiviral T cells. *Nature* 2012; 481: 394–398.
47. Lang PA, Lang KS, Xu HC *et al.* Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting $CD8^+$ T-cell immunity. *Proc Natl Acad Sci U S A* 2012; 109: 1210–1215.
48. Narni-Mancinelli E, Jaeger BN, Bernat C *et al.* Tuning of natural killer cell reactivity by NKP46 and helios calibrates T cell responses. *Science* 2012; 335: 344–348.
49. Andrews DM, Estcourt MJ, Andoniou CE *et al.* Innate immunity defines the capacity of antiviral T cells to limit persistent infection. *J Exp Med* 2010; 207: 1333–1343.
50. Luci C, Reynders A, Ivanov II *et al.* Influence of the transcription factor $ROR\gamma t$ on the development of $NKP46^+$ cell populations in gut and skin. *Nat Immunol* 2009; 10: 75–82.
51. Luci C, Gaudy-Marqueste C, Rouzaire P *et al.* Peripheral natural killer cells exhibit qualitative and quantitative changes in patients with psoriasis and atopic dermatitis. *Br J Dermatol* 2012; 166: 789–796.
52. Cella M, Fuchs A, Vermi W *et al.* A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* 2009; 457: 722–725.
53. Ashkar AA, Rosenthal KL. Interleukin-15 and natural killer and NKT cells play a critical role in innate protection against genital herpes simplex virus type 2 infection. *J Virol* 2003; 77: 10168–10171.
54. Thapa M, Kuziel WA, Carr DJ. Susceptibility of CCR5-deficient mice to genital herpes simplex virus type 2 is linked to NK cell mobilization. *J Virol* 2007; 81: 3704–3713.
55. Hall LJ, Clare S, Dougan G. NK cells influence both innate and adaptive immune responses after mucosal immunization with antigen and mucosal adjuvant. *J Immunol* 2010; 184: 4327–4337.
56. Middendorp S, Nieuwenhuis EE. NKT cells in mucosal immunity. *Mucosal Immunol* 2009; 2: 393–402.
57. Lindqvist M, Persson J, Thorn K, Harandi AM. The mucosal adjuvant effect of α-galactosylceramide for induction of protective immunity to sexually transmitted viral infection. *J Immunol* 2009; 182: 6435–6443.