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Mucosal Luminal Manipulation of T Cell Geography Switches on Protective Efficacy by Otherwise Ineffective Parenteral Genetic Immunization

Michael Santosuosso, Sarah McCormick, Elizabeth Roediger, Xizhong Zhang, Anna Zganiacz, Brian D. Lichty, and Zhou Xing

Genetic immunization holds great promise for future vaccination against mucosal infectious diseases. However, parenteral genetic immunization is ineffective in control of mucosal intracellular infections, and the underlying mechanisms have remained unclear. By using a model of parenteral i.m. genetic immunization and pulmonary tuberculosis (TB), we have investigated the mechanisms that determine the failure and success of parenteral genetic immunization. We found that lack of protection from pulmonary Mycobacterium tuberculosis (M.tb) challenge by i.m. immunization with a recombinant adenovirus-vectorized tuberculosis vaccine was linked to the absence of M.tb Ag-specific T cells within the airway lumen before M.tb challenge despite potent T cell activation in the systemic compartments. Furthermore, pulmonary mycobacterial challenge failed to recruit CD8 T cells into the airway lumen of i.m. immunized mice. Such defect in T cell recruitment, intra-airway CTL, and immune protection was restored by creating acute inflammation in the airway with inflammatory agonists such as virus. However, the Ag-specific T cells recruited as such were not retained in the airway lumen, resulting in a loss of protection. In comparison, airway exposure to low doses of soluble M.tb Ags not only recruited but retained Ag-specific CD8 T cells in the airway lumen over time that provided robust protection against M.tb challenge. Thus, our study reveals that mucosal protection by parenteral immunization is critically determined by T cell geography, i.e., whether Ag-specific T cells are within or outside of the mucosal lumen and presents a feasible solution to empower parenteral immunization strategies against mucosal infectious diseases. The Journal of Immunology, 2007, 178: 2387–2395.
to its robust protective efficacy following airway mucosal administration (9).

Materials and Methods

Mice

Six- to 10-wk-old female BALB/c mice were purchased from Harlan Laboratories. Congenic BALB/c mice were purchased from Jackson ImmunoResearch Laboratories. All mice were housed in a specific pathogen-free level B facility. All experiments were conducted in accordance with the animal ethics research board of McMaster University.

Recombinant viral vaccines

An adenoviral vector expressing Ag85A was created as described previously (10). Briefly, Ag85A sequence was amplified from M. tb genomic sequences and was then ligated into a vector that contains a human tissue plasminogen activator (tPA) signal peptide. The tPA-Ag85A sequence was then cloned into a shuttle vector between a CMV promoter and a SV40 poly(A) signal in orientation. This plasmid was cotransfected with a rescue plasmid pHBG10 into 293 cells (18, 19). Recombinant replication-deficient Ad was then rescued by homologous recombination. To develop a recombinant vesicular stomatitis virus (VSV) vaccine expressing Ag85A (VSVAg85A), the CDN encoding Ag85A and human tPA signal peptide was PCR amplified. The PCR product was digested with Xhol and Nhel, purified, and ligated into pVSV-XNPD (20) to create pVSV-XNPD Ag85A-tPA plasmid. The plasmid was cotransfected with the helper plasmid pCMV ΔE1 ΔE3 to rescue VSVAg85A virus, which was subsequently amplified and purified following standard protocols (20). The production of Ag85A from VSVAg85A-infected cells was confirmed by Western immunoblotting using an Ag85A mAb as described previously (10).

Immunization

Mice were immunized with a dose of 5 × 10⁷ PFU of AdAg85A (~2.5 × 10⁷ viral particles) via either an i.n. or i.m. route (9). Briefly, anesthetized mice were either injected once in each quadriceps with 25 μl of PBS for a total of 50 μl containing 5 × 10⁷ PFU of AdAg85A or were allowed to slowly breathe in 25 μl of PBS containing 5 × 10⁷ PFU of AdAg85A. In some experiments, 1 wk post-AdAg85A i.m. immunization, mice received a total of 50 μl of PBS containing 5 × 10⁷ PFU of AdAg85A or were allowed to slowly breathe in 25 μl of PBS containing 5 × 10⁷ PFU of AdAg85A. In some experiments, 1 wk post-AdAg85A i.m. immunization, mice received 5 × 10⁷ PFU of Addl70.3 (empty vector) or 50 μg of unmethylated CpG oligonucleotides via the i.n. route as described above. In separate experiments, 2 wk post-i.m. AdAg85A immunization Ag85A complex protein was administered i.n. every week for a total of 6 wk (see Fig. 7A).

Mycobacterium challenge and colony forming assay

Mycobacterium bovis bacillus Calmette-Guérin (BCG) (Connaught strain) and M. tb (H37Rv strain) (ATCC 27294) were grown in Middlebrook 7H broth supplemented with Middlebrook oleic acid-albumin-dextrose-catalase enrichment (Invitrogen Life Technologies), 0.002% glycerol, and 0.05% Tween 80 for 10–15 days then aliquoted and stored in −70°C until needed (21). Before each use, BCG or M. tb bacilli were washed with PBS containing 0.05% Tween 80 twice and passed through a 22-μm needle 10 times to disperse clumps. For the challenge study with BCG, 2 × 10⁶ CFU of live BCG bacilli were administered via the intratracheal route in 40 μl of PBS, as described previously (9). BCG-infected mice were sacrificed 21 and 28 days postchallenge, and cells were isolated and stimulated as described in the following section. For M. tb infection, immunized and nonimmunized mice were infected i.n. with 10,000 CFU of M. tb (at the indicated time points following immunization in the level III contained facility of McMaster University). The level of bacterial burden was determined at the described time points in the lung and spleen by plating serial dilutions of tissue homogenates in triplicates onto Middlebrook 7H10 agar plates containing Middlebrook oleic acid-albumin-dextrose-catalase enrichment (21). Plates were incubated at 37°C for 21 days in semisealed plastic bags. Colonies were then counted, calculated, and presented as log_{10} CFU per organ.

Cell isolation

Immunized BALB/c mice were sacrificed at the time points indicated within each experiment. Spleens and lungs were removed aseptically, and the intra-airway luminal cells were removed from the lung by exhaustive lavage as described previously (22). Briefly, the mouse lung was lavaged five times to a volume of 1.8 ml of PBS through a polyethylene cannulated into the trachea to ensure maximal recovery. After lavage, the lungs were perfused through the left ventricle with Hank’s buffer to remove RBC from the vasculature. The lungs were then cut into small piece (1 × 1 mm) and incubated with collagenase type 1 (Sigma-Aldrich) for 1 h at 37°C. Lung fragments were then washed with a 100-μm filter. Cells were collected and enumerated on a hemocytometer after dilution in Turks Counting buffer. Spleen cells were isolated as described previously (9). All isolated cells (spleen, lung, and airway-lumen) were then resuspended in RPMI 1640 medium supplemented with 5% FBS and 100 μg/ml penicillin and streptomycin.

FACS, intracellular cytokine staining (ICCS), and tetramer staining

Single-cell suspension from immunized mice of spleen, lung, and airway-luminal cells were obtained as described above. Cells were cultured in a 150-bottom 96-well plate at a concentration of 20 × 10⁶ cell/ml, and airway-lumen-derived cells were cultured at a concentration of 5 × 10⁶ cell/ml. Cells are cultured in the presence of Golgi plug (5 μg/ml brefeldin A; BD Pharmingen) and either no stimulation, Ag85A-specific CD4 (LTSELP WLQANRHVKPTGS) or CD8 (MPVGGQSSST) T cell peptides at a concentration of 1 μg/ml, or mycobacterium culture filtrate and Ag85 complex peptide at a concentration of 2 μg/ml (Colorado State University) for 1 h (17). Cells are then washed and blocked with CD16/CD32 in 0.5% BSA/PBS for 15 min on ice and then stained with the appropriate surface Abs. Cells are then washed, permeabilized, and stained according to the manufacturer’s instructions included in the ICCS kit (BD Pharmingen).

In vivo intratracheal CTL assay

The in vivo intratracheal CTL assay was conducted as described previously (9). Briefly, CTL target cells were prepared from the spleen of naive BALB/c mice and pulsed with Ag85A-CD8 peptide (10 μg/ml) or no peptide overnight at 4°C. Peptide-pulsed splenocytes were then labeled with a high concentration of CFSE (5 μM) or unpulsed splenocytes with a low concentration (0.5 μM) of CFSE. Splenocytes were then washed and enu- merated. Before adoptive transfer, 2.5 × 10⁶ pulsed and 2.5 × 10⁶ unpulsed control splenocytes (1:1 ratio) were combined at a final volume of 40 μl of PBS and transferred intratracheally to immunized mice. Mice were then sacrificed at 5 h after the adoptive transfer, the airway lumen were isolated as described above by bronchoalveolar lavage. Cells were then run on the LSR II (BD Pharmingen) for the assessment of percentage of CFSE-labeled target cells. The percentage of in vivo loss of CFSE-labeled target cells was determined as the loss of such cells after in vivo incubation relative to CFSE- labeled unpulsed control splenocytes and thus taken as the measure of CTL. It was calculated by using the formula: percentage of killing = (1 – (unpulsed cells/peptide pulsed cells from unimmunized control) × (unpulsed/peptide pulsed from immunized group)) × 100.

Results

Impaired recruitment of Ag-specific T cells into the airway lumen and lack of protection by i.m. AdAg85A immunization against mycobacterial challenge

To demonstrate the efficacy of parenteral genetic vaccination against a mucosal infectious agent, we immunized BALB/c mice with AdAg85A i.m., and then 4 wk later, we challenged mice with 10,000 CFU of M. tb via the airway. Indeed, mice immunized i.m. with AdAg85A, different from those i.m. immunized, were not protected against pulmonary M. tb challenge (Table I), which is consistent with our previous findings (9, 10, 17).

In contrast to the airway luminal recruitment and retention of T cells seen in well-protected, i.m. immunized mice, we previously found that i.m. immunized mice lacked Ag-specific T cells within the airway lumen during the first 5 days after mycobacterial challenge (9, 17). This suggests that Ag-specific T cells need to be present in the airway lumen around the time...
Impaired recruitment of Ag85A-specific CD8 T cells in i.m.
immunized mice after mycobacterial challenge. BALB/c mice were
immunized with 5 \times 10^7 PFU of AdAg85A either i.m. (IM) or i.n. (IN). Eight weeks later mice were
challenged intratracheally with 1 \times 10^8 PFU of recombinant repli-
cating VSV-expressing Ag85A (VSVAg85A). Lungs were removed, ho-
genized, and determined for the level of VSV infection by viral PFU
assay at 3 days post-VSV challenge. Data are representative of two inde-
pendent experiments and expressed as mean value ± SEM of five mice per
group. *** p < 0.005 as compared with all other groups.

**FIGURE 1.** Impaired recruitment of Ag85A-specific CD8 T cells in i.m.
immunized mice after mycobacterial challenge. BALB/c mice were immu-
nized with 5 \times 10^7 PFU of AdAg85A either i.m. (IM) or i.n. (IN). Eight weeks later mice were
challenged intratracheally with 1 \times 10^8 PFU of recombinant replic-
cating VSV-expressing Ag85A (VSVAg85A). Lungs were removed, ho-
genized, and determined for the level of VSV infection by viral PFU
assay at 3 days post-VSV challenge. Data are representative of two inde-
pendent experiments and expressed as mean value ± SEM of five mice per
group. *** p < 0.005 as compared with all other groups.

**FIGURE 2.** Inability of parenteral genetic immunization to protect from
acute respiratory viral infection. BALB/c mice were immunized with 5 \times 10^7 PFU of AdAg85A either i.m. (IM) or i.n. (IN). Eight weeks later mice were
challenged intratracheally with 1 \times 10^8 PFU of recombinant repli-
cating VSV-expressing Ag85A (VSVAg85A). Lungs were removed, ho-
genized, and determined for the level of VSV infection by viral PFU
assay at 3 days post-VSV challenge. Data are representative of two inde-
pendent experiments and expressed as mean value ± SEM of five mice per
group. *** p < 0.005 as compared with all other groups.

**FIGURE 3.** Recruitment of Ag85A tetramer-specific CD8 T cells to the
airway lumen of i.m. immunized mice by nonspecific inflammatory ago-
nists. BALB/c mice were immunized with 5 \times 10^7 PFU of AdAg85A i.m.
(IM) with or without i.n. (IN) administration of 5 \times 10^7 PFU of inflam-
mafion agonist Add70.3. Some mice were immunized with 5 \times 10^7 PFU of
AdAg85A i.m. (IN). All mice were sacrificed at 2 wk postimmunization. Tetramer-positive CD8 T cells in BAL (A) or the spleen (B) were quanti-
fied by immunostaining and FACS analysis. Data are expressed as mean value ± SEM of four mice per group, representative of two independent
experiments. *, p < 0.05; ***, p < 0.005 as compared with AdAg85A i.m.
group.
whether lack of protective responses after parenteral genetic immunization may also apply to acute mucosal viral infections to which protective T cell activation and responses occur within a few days (23, 24). To examine this, we immunized BALB/c mice i.m. with AdAg85A, and 8 wk later, we intratracheally challenged these mice with a recombinant replication-competent VSV that was engineered to express Ag85A (VSVAg85A) and analyzed VSV viral titers in the lungs 3 days post-VSV challenge. We found that similar to the lack of protection from mycobacterial challenge, mice i.m. immunized with AdAg85A were not protected from VSVAg85A challenge (Fig. 2), in contrast to a great level of protection observed in i.n. immunized mice. These results suggest that the lack of protective immune responses at the airway mucosa following parenteral genetic immunization is true both for intracellular bacterial and viral infectious agents. Taken together, our findings point to the importance of the presence of Ag-specific T cells right at the site of exposure before infection.

Inflammatory recruitment into the airway lumen of i.m. AdAg85A-immunized mice by nonspecific immune agonists. BALB/c mice were immunized with 5 × 10⁷ PFU of AdAg85A i.m. (IM) with or without i.n. (IN) administration of 5 × 10⁷ PFU of proinflammatory agonist Add170.3 at 1 wk postimmunization. Some mice were immunized i.n. with AdAg85A. At 2 wk postimmunization, some mice were sacrificed, and Ag-specific, IFN-γ-secreting CD8 T cells (A) and CD4 (B) were analyzed by ICCS or received CD8 T cell peptide-pulsed, CFSE-labeled target cells intratracheally and subsequently analyzed for in vivo intratracheal CTL activities (C and D). Histograms shown in D are representative of the loss of CFSE-labeled, peptide-pulsed splenocyte targets with the right and left peaks being CFSE-labeled Ag85A peptide-pulsed splenocytes and unpulsed splenocytes, respectively. Other mice were challenged via the airway with virulent M.tb and sacrificed 4 wk post-M.tb challenge for the level of M.tb infection in the lung (E). Data are expressed as the mean ± SEM of four mice per group (A and B), three mice per group (C and D), and seven mice per group (E). All data are representative of two independent experiments. * p < 0.05; ** p < 0.01; *** p < 0.005, as compared with naive or AdAg85A i.m. groups.
lumen upon airway *M. tb* exposure. To investigate the mechanisms required for such systemically activated T cells to be recruited into the airway lumen, we set out to examine the effect of nonspecific acute inflammatory agonists. To this end, BALB/c mice were immunized i.m. with AdAg85A and 1 wk later were exposed i.n. to an empty Ad (Addl70.3) or unmethylated CpG oligonucleotides. T cell responses in the airway lumen (bronchoalveolar lavage (BAL)), lung, and spleen were examined 2 wk postimmunization. We observed a remarkably enhanced number of Ag85A tetramer-specific CD8 T cells in the airway lumen of i.m. immunized mice after Addl70.3 delivery, and this level of T cell responses rose to that by i.n. immunization, in contrast to the lack of such airway T cells in i.m. immunized mice without exposure to an acute inflammatory agonist (Fig. 3A). Of interest, this marked inflammatory recruitment of tetramer-specific CD8 T cells into the airway lumen was accompanied by a concurrent decrease of such CD8 T cells in the spleen (Fig. 3B), suggesting a systemic contribution to the T cells recruited into the airway. In addition to the effect of Addl70.3, a very similar outcome was observed when Addl70.3 was replaced with a different proinflammatory agent CpG oligonucleotides (data not shown). These results suggest that Ag-specific T cells systemically activated by parenteral genetic immunization can be recruited to the airway lumen by nonspecific immune stimuli with proinflammatory properties.

**IFN-γ production, CTL and immune protection by Ag-specific T cells in the airway lumen recruited by nonspecific inflammatory agonists**

To investigate whether parenteral immunization-activated, Ag85A tetramer-specific T cells recruited into the airway lumen by inflammatory agents were capable of functional activities, we first examined whether the recruited CD8 T cells could produce IFN-γ. BALB/c mice were immunized i.m. with AdAg85A and 1 wk later were exposed i.n. to Addl70.3, and Ag-specific, IFN-γ-producing CD8 T cells in the airway lumen (BAL) were examined by ex vivo Ag stimulation and ICCS 2 wk postimmunization. The number of IFN-γ-producing CD8 T cells in BAL of i.m. immunized mice was dramatically increased by exposure to Addl70.3, which was even greater than that by i.n. immunization (Fig. 4A). The same was true of Ag-specific, IFN-γ-positive CD4 T cells (Fig. 4B). We next examined whether the recruited CD8 T cells could kill Ag-pulsed targets via their CTL activities by using an in vivo intratracheal CTL assay we previously developed to measure CD8 T cell-mediated loss of CFSE-labeled, Ag85A peptide-pulsed target cells in the airway (9). Mice were immunized with AdAg85A i.m., and 1 wk later, Addl70.3 was administered i.n.: 2 wk postimmunization, mice were injected intratracheally with Ag85A C8 T cell peptide-pulsed CTL target cells. While there was a minimal Ag85A-specific CTL killing in the airway lumen of i.m. AdAg85A mice, it was enhanced markedly by pretreating i.m. AdAg85A-immunized mice with an inflammatory agonist Addl70.3 (Fig. 4, C and D), the level of which was comparable to that observed in the lung of i.n. immunized mice.

To further understand the function of T cells recruited into the airway lumen, we examined the immune protective property of these cells. Thus, i.m. parenterally AdAg85A-immunized mice were treated i.n. with Addl70.3 at 1 wk, and at 2 wk postimmunization, they were challenged with *M. tb* via the airway. These mice were then sacrificed at 4 wk post-*M. tb* challenge. While i.m. AdAg85A mice were not protected at all, as we have previously demonstrated (9, 10), i.m. AdAg85A mice pretreated with an inflammatory agonist Addl70.3 were much better protected and such level of protection was near that accomplished by i.n. mucosal AdAg85A immunization (Fig. 4E). These results demonstrate that

parenterally activated Ag-specific T cells recruited into the airway lumen by nonspecific inflammatory signals are fully capable of IFN-γ production, CTL, and immune protection against a subsequent airway *M. tb* challenge.

**Recruited Ag-specific T cells by inflammatory agonists cannot be retained in the airway lumen over time**

The above observations indicate that the Ag85A-specific T cells systemically activated by parenteral genetic immunization could be brought into the airway lumen nonspecifically by an acute inflammatory insult of viral or CpG nature. This suggests that these T cells moved into the airway luminal space from systemic tissue compartments, including the lung interstitium and spleen, likely in response to acutely enhanced expression of chemokines and adhesion molecules in the lung (23, 24). We next asked the question as to whether these T cells recruited as such would persist within the airway lumen. Persistence of memory T cells at the mucosal site of pathogen entry is considered critical to vaccination (1, 8). To approach, we immunized BALB/c mice with AdAg85A i.m., and 1 wk later, we administered Addl70.3 i.n.; 8 and 12 wk postimmunization airway luminal T cells were examined. We found that levels of Ag85A tetramer-positive CD8 T cells in the airway lumen of i.m. AdAg85A-immunized, Addl70.3-treated mice dropped back close to the levels observed in i.m. AdAdd85A mice without inflammatory treatment (Fig. 5A). In comparison to those in the airway lumen, the numbers of tetramer-positive CD8 T cells remained comparable in the systemic tissue compartments such as the spleen between AdAg85A i.m., AdAg85A i.m. plus Addl70.3 i.n., and AdAg85A i.n. groups (Fig. 5B). These results suggest that systemically activated Ag-specific T cells, particularly CD8 T cells that are recruited into the airway lumen by nonspecific inflammatory agonists, fail to persist in the airway lumen for extended periods of time.

**Loss of recruited Ag-specific CD8 T cells in the airway lumen leads to diminished IFN-γ production, CTL, and immune protection**

Having demonstrated that the recruited Ag-specific CD8 T cells were not retained in the airway lumen at later times after the initial inflammatory recruitment, we examined whether the loss of Ag-specific CD8 T cells would lead to the loss of T cell functions,
including IFN-γ secretion, airway luminal CTL activities, and immune protection from M.tb challenge. Thus, we immunized BALB/c mice i.m. with AdAg85A and 1 wk later administered Addl70.3 i.n. At 8 and 12 wk postimmunization, we first examined Ag-specific, IFN-γ-producing CD8 T cells (A) and CD4 T cells (B) in BAL by ICCS. Indeed, in accord with sharply diminished tetramer-positive CD8 T cells in BAL (Fig. 5A), the number of Ag-specific, IFN-γ-positive CD8 T cells in the airway lumen also markedly decreased (Fig. 6A). Of note, the number of Ag-specific CD4 T cells in the airway lumen of Addl70.3-treated mice remained stable (Fig. 6B). To examine CTL activities, the mice were treated as above, and at 8 wk postimmunization, mice were injected intratracheally with peptide-pulsed CTL target cells for examination of in vivo intratracheal CTL-mediated killing activities. We found that the loss of Ag-specific CD8 T cells in the airway lumen was accompanied by a complete loss of Ag-specific CTL killing of the targets (Fig. 6, C and D). Upon examination of immune protection at 4 wk post-M.tb challenge (challenge at 8 wk postimmunization), the loss of CD8 T cells and CTL from the airway lumen was found to result in loss of protection against pulmonary M.tb infection (Fig. 6E). These results indicate that airway luminal functionally activated CD8 T cells play an important role in the observed protection.

Exogenously administered soluble M.tb Ags can recruit and retain Ag-specific CD8 T cells in the airway lumen following i.m. AdAg85A immunization

Considering our findings that airway exposure to nonspecific acute inflammatory agents could recruit, but not retain, Ag-specific T cells in the airway lumen and the notion that in certain models the
FIGURE 7. Recruitment and retention of Ag85A tetramer-specific CD8 T cells in the airway lumen by airway administration of soluble Ag85 complex proteins in i.m. immunized mice. BALB/c mice were immunized with 5 × 10^7 PFU of AdAg85A i.m. Two weeks later 2.5 μg of soluble Ag85A complex proteins was administered i.n. weekly for a total of 6 wk as depicted in the diagram (A). Some mice were sacrificed at 1 (B) or 6 (C) wk following the final administration of Ag85 proteins for analysis of airway luminal (BAL) Ag85A tetramer-specific CD8 T cells by immunostaining and FACS. Data are expressed as mean value ± SEM of three mice per group, representative of two independent experiments. *, p < 0.05; **, p < 0.01 as compared with all other groups.

FIGURE 8. Immune protection by Ag85A-specific T cells retained within the airway lumen by airway administration of soluble Ag85 complex proteins in i.m. immunized mice. BALB/c mice were treated as in Fig. 7, and at 6 wk after the last administration of soluble Ag85 complex proteins, mice were challenged via the airway with virulent M. tb and sacrificed 4 wk post-M. tb challenge as depicted in the diagram (A) and analyzed for the level of M. tb infection in the tissue (B). Data are expressed as mean value ± SEM of five mice per group. ***, p < 0.005 as compared with naive and AdAg85A i.m. groups.

Recruited and retained Ag-specific T cells in the airway lumen by exogenous soluble M. tb Ags confer robust protection against pulmonary M. tb challenge

Having demonstrated that exogenously administered soluble M. tb Ags were able to recruit and retain Ag-specific T cells in the airway lumen, we examined whether the retained cells were able to protect against pulmonary M. tb challenge. To this end, we immunized BALB/c mice i.m. with AdAg85A, and 2 wk later, we administered a small amount of purified soluble Ag85 complex proteins i.n. once weekly for 6 wk; the mice then rested for 6 wk before they were challenged with M. tb i.n. for immune protection assay (Fig. 8A). The level of M. tb infection was examined 4 wk post-M. tb challenge. We observed that the retained Ag-specific T cells in the airway lumen of i.m. immunized mice were able to protect against M. tb challenge to a similar degree as that by i.n. mucosal immunization (Fig. 8B). Thus, respiratory exposure to nonimmunogenic soluble M. tb Ags fully restores immune protection in parenterally immunized hosts that are otherwise unprotected.

Discussion

The majority of currently successful human vaccines has been administered parenterally to the muscle or skin, and these vaccines protect via induction of protective Ab responses. There is a lack of effective vaccines for mucosal infectious diseases such as AIDS, TB, and herpes virus infection, and the immunity for these infections depends largely on protective memory T cell responses (1–3). While genetic-based vaccines represent a new generation of promising vaccines for such mucosally transmitted diseases, these vaccines when administered parenterally fail to confer effective and lasting immune protection at the mucosal site (5–10). The mechanisms underlying this phenomenon have remained largely unknown, and there is an urgent need to understand what determines the failure and success of parentential genetic immunization in protection from mucosal infectious disease.
We have recently demonstrated that respiratory mucosal immunization leads to the induction and retention of Ag-specific T cells in the airway lumen capable of immune protection from pulmonary M.tb challenge (9). In contrast, while parenteral i.m. immunization induces a high frequency of activated Ag-specific T cells systemically, it is unable to induce airway luminal T cells and immune protection. In fact, we found that these systemically activated T cells by parenteral immunization did not have any defect in their immune protective property because they could protect against pulmonary M.tb challenge when adoptively transferred into the airway lumen of naive SCID hosts (9). In our current study, we found that the lack of protection from pulmonary M.tb challenge by parenteral immunization was associated not only with the absence of M.tb Ag-specific T cells within the airway lumen before M.tb challenge but also with the failure of sufficient Ag-specific T cell recruitment into the airway lumen after mycobacterial exposure. While airway administration of proinflammatory agents such as virus or CpG could recruit functionally capable T cells into the airway lumen and restore immune protection, these cells, particularly CD8 T cells, and associated protection could persist in the airway lumen only for a limited period of time. Of interest, the Ag-specific CD4 T cells brought into the airway lumen by acute inflammation sustained with time, different from CD8 T cell counterparts, suggesting a critical protective role by airway luminal CD8 T cells and perhaps a different mechanism by which CD4 and CD8 T cells are maintained in the airway. Compared with the effect of nonspecific inflammatory agonists, airway exposure to nonimunogenic soluble M.tb Ags recruited and retained Ag-specific T cells in the airway lumen, which were capable of robust protection against pulmonary M.tb challenge.

Furthermore, our current findings suggest that in addition to protection against intracellular mycobacterial infection, airway luminal T cells are also important to protection against acute viral infection. This conclusion is also supported by the evidence generated in models of primary and secondary respiratory viral infection (27–29). Indeed, it has been suggested that the presence of mucosal luminal T cells is predictive of quicker secondary immune responses (9, 26, 29, 30). Ely et al. (31) have also observed that secondary respiratory influenza infection could transiently recruit Sendai virus-specific CD8 T cells into the airway lumen, following primary respiratory Sendai viral infection. Thus, these observations together suggest that irrespective of the route of primary immune activation, inflammatory signals in the airway may induce the recruitment of T cells of irrelevant Ag specificity into the airway lumen. Further to these findings, we found that following proinflammatory provocation to parenterally immunized hosts, the influx and accumulation of Ag-specific CD8 T cells was transient compared with relatively sustained Ag-specific CD4 T cells, suggesting a differential mechanism for the maintenance of CD8 and CD4 T cells in the airway. These findings suggest a critical role by airway CD8 T cells in immune protection against pulmonary TB, following genetic vaccination. Of prominent significance is our finding that different from the effect of acute inflammatory agonists, airway exposure to soluble nonimmunogenic proteins of the same antigenic specificity to the vaccine Ag could not only recruit but retain Ag-specific T cells within the airway lumen. Such mode of manipulation and modulation to T cell geography across the mucosal membrane successfully turns a parenterally immunized and otherwise unprotected host into the one that is as well protected from a mucosal infectious challenge as a mucosally immunized host. Our findings thus indicate suggest that airway Ag exposure plays a critical role in recruiting and retaining systemically activated Ag-specific T cells in the mucosal luminal space.

The mechanisms of T cell recruitment and subsequent retention within the airway lumen by soluble M.tb Ag exposure in our model remain to be completely understood. As we found that the soluble M.tb Ag used in our study by itself was not immunogenic and incapable of T cell activation in naive hosts, the local signals required for mobilizing the T cells primed in the systemic compartments by prior parenteral genetic immunization are likely different from those required for activating naive T cells. By using a model of primary respiratory Sendai virus infection model, Ely et al. (32) have recently reported that the maintenance of virus-specific CD8 T cells in the airway requires the continuous recruitment of circulating cells. In contrast, by using a primary influenza virus infection model, Zammit et al. (25) found that the maintenance of such virus-specific memory CD8 T cells involved the continuing Ag presentation in the local draining lymph nodes. Our model differs much from these primary respiratory viral infection models in that it begins with a completely naive local airway environment and a systemically primed immune system. It is possible that, in our model, airway soluble M.tb Ag exposure acts to recruit and maintain systemically activated T cells in the airway by several mechanisms, including the generation of signals for T cell migration and proliferation. The sources of such T cells could be from both the lung interstitial pool and systemic lymphoid tissues such as the spleen. Indeed, we found the presence of Ag-specific T cells both in the lung interstitium and spleen and a relationship between the influx of CD8 T cells to the airway lumen and decreased numbers of Ag-specific CD8 T cells in the spleen. It is likely that the maintenance of such T cells in the airway lumen involves both Ag presentation and T cell proliferation, which may happen both in the bronchial lymphoid aggregates and draining lymph nodes because soluble Ags picked up by airway mucosal APC may activate T cells in both sites (33).

As parenteral administration represents a convenient and safe way to deliver vaccines, the majority of current human vaccines has been administered via the muscle or skin to successfully induce protective humoral immune responses. It is believed that the parenteral route will also be the route of choice in the future for administering the novel vaccines designed to fight against mucosal infectious diseases such as HIV and TB. The challenge is how to make such immunization successful in triggering long-lasting protective memory T cell responses at the mucosal site of pathogen entry. Our current studies have revealed that the failure or success of parenteral immunization hinges critically on T cell geography, i.e., whether or the Ag-specific T cells are within or outside of the mucosal lumen at a right time in order for immune protection to occur. Thus, parenteral genetic immunization fails to induce mucosal luminal T cells, which are critically required for immune protection at the time of mucosal pathogen challenge. However, we find that systemically activated memory T cells by parenteral immunization can be most ideally mobilized into the airway lumen, present for prolonged periods of time, by brief exposures to soluble and harmless Ags. These findings thus suggest that administering limited doses of soluble Ags to the mucosal site represents a solution to empower parenteral genetic vaccination strategies against mucosal intracellular infectious disease.

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Disclosures

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