The development of mucosal vaccines for both mucosal and systemic immune induction and the roles played by adjuvants

Vaccination is the most successful immunological practice that improves the quality of human life and health. Vaccine materials include antigens of pathogens and adjuvants potentiating the effectiveness of vaccination. Vaccines are categorized using various criteria, including the vaccination material used and the method of administration. Traditionally, vaccines have been injected via needles. However, given that most pathogens first infect mucosal surfaces, there is increasing interest in the establishment of protective mucosal immunity, achieved by vaccination via mucosal routes. This review summarizes recent developments in mucosal vaccines and their associated adjuvants.

Keywords: Adjuvants, Mucosal immunity, Vaccines

Introduction

Early vaccination strategies used live or attenuated pathogens to induce adaptive immunity [1]. However, the re-activation of attenuated vaccine materials and the possible tumorigenicity of inactivated oncogenic viruses are important safety concerns [2]. Thus, non-pathogenic materials including inactivated toxins, synthetic peptides, and recombinant subunit proteins have been considered as vaccine materials [3]. However, these antigens are poor in immunogenicity and must be given with supplemental materials to potentiate the vaccination capacity [3]. Such supplemental materials are termed adjuvants and can be divided into two classes depending on their mode of action: efficient delivery of vaccine materials and/or stimulation of the immune system (Tables 1, 2) [1]. Adjuvants facilitating vaccine delivery include liposomes, nanogels, oil-in-water emulsions, and virosomes targeting the co-administered antigens to professional antigen-presenting cells (APCs) [4]. Adjuvants that stimulate the immune system include molecules binding to intracellular receptors including Toll-like receptors (TLRs), Nod-like receptors, and RIG-I-like receptors and to cytosolic DNA sensors, all of which modulate the immune response [1]. However, most adjuvants have been evaluated in the context of parenteral immunization; thus, it is not clear how well the adjuvants function in the mucosal immune compartment.

The Mucosal Immune System

Mucosal surfaces cover 400 m² of the body including the gastrointestinal, urogenital,
and respiratory tracts [5]. Mucosae are continuously exposed to microbiota and antigens. The gastrointestinal mucosa is especially prone to the development of tolerogenic microenvironments, where luminal antigens may persist. The mucosal immune system has both inductive and effector sites differing in terms of their anatomical and functional characteristics [6]. The major mucosal immune inductive sites include gut-associated lymphoid tissue (GALT) and the nasopharyngeal-associated lymphoid tissue (NALT). GALT includes Peyer’s patches, mesenteric lymph nodes, and isolated lymphoid follicles, while NALT includes tonsils/adenoids, inducible bronchus-associated lymphoid tissue, cervical lymph nodes, and hilar lymph nodes. Mucosal immune inductive sites are covered by follicle-associated epithelium (FAE), which is composed of enterocytes and M cells.

M cells are specialized epithelial cells for antigen uptake [7]. These cells are overlaid by a thin mucus layer and possess short irregular microvilli [8]. M cells can transfer antigens via transcytosis to APCs located in pockets within M cell clusters [9]. Dendritic cells that come in contact with antigens transcytosed through M cells enter the interfollicular T cell zone to activate naïve T cells [10]. Finally, effector T cells move to the B cell follicles of germinal centers (GCs) and secrete cytokines capable of promoting IgA class-switch recombination [11]. In mucosal immune effector sites such as the lamina propria of the gut, the upper respiratory tract, and the female reproductive tract, IgA+ plasma cells terminally differentiate to release secretory IgA (SlgA), the most important immune effector molecule in the mucosa. SlgA is transported across mucosal epithelial cells via a polymeric Ig receptor (pIgR) (Fig. 1) [12]. SlgA is a major immune effector at mucosal surfaces that acts via three mechanisms: antigen excretion, immune exclusion, and intracellular antigen neutralization (Fig. 2) [13]. Antigen excretion by SlgA features the binding of SlgA to pathogen-derived antigens, thus inhibiting pathogen–epithelial cell contact. SlgA exerts immune exclusion by eliminating antigens via secretion of an IgA–antigen complex, and invading pathogens can also be eliminated by complex formation with IgA-joining (J) chain-plgR. SlgA inhibits the binding of pathogens and/or pathogenic antigens to specific receptors by neutralizing and eventually removing the pathogenic antigens.

| Table 1. Currently licensed adjuvants used as carriers of vaccine materials |
|--------------------------------|------------------|-----------------|--------------------------------------------------|
| Adjuvant name | Adjuvant class | Immune response | Component |
| Alum | Mineral salts | Antibody, Th2 response | Aluminum phosphate or aluminum hydroxide |
| MF59 | Oil-in-water emulsion | Antibody, Th1/Th2 response | Squalene, Polysorbate 80 (Tween 80), sorbitan trioleate (Span 85) |
| Virosomes | Liposomes | Antibody, Th1/Th2 response, cross-presentation | Lipids, hemagglutinin |
| AS03 | Oil-in-water emulsion | Antibody, Th1/Th2 response | Squalene, Polysorbate 80 (Tween 80), α-tocopherol |
| Montanide ISA51 | Water-in-oil emulsion | Antibody, Th1/Th2 response | Drakeol 6 VR, mannide monooleate |

Based on Rappuoli R et al. Nat Rev Immunol 2011;11:865-72 [1].

| Table 2. Immunostimulatory molecules used as vaccine adjuvants |
|--------------------------------|------------------|-----------------|--------------------------------------------------|
| Adjuvant name | Target receptor | Type (component) | Immune response |
| Licensed adjuvant |
| RC529 | TLR4 | RC529 | Antibody, Th1 response |
| AS01 | TLR4 | Liposome, MPL, OS21 | Antibody, Th1 response, CD8+ T cells |
| AS04 | TLR4 | Aluminum hydroxide, MPL | Antibody, Th1 response |
| Not licensed adjuvant |
| Poly(I:C), Poly(I):C(LC) | TLR3 | dsRNA | Type I IFN, pro-inflammatory cytokines, antibody, CD4/CD8 response |
| Imiquimod, Resiquimod, Gardiquimod | TLR7/TLR8 | ssRNA | Type I IFN, pro-inflammatory cytokines, antibody, CD4/CD8 response |
| IC31 | TLR9 | Unmethylated CpG DNA | Type I IFN, pro-inflammatory cytokines, antibody, CD8 response |
| iE-DAP, MDP | NOD1/2 | Peptidoglycan | Pro-inflammatory cytokines, antibody |
| MB, defective interfering (DI) RNA | RIG-I, MDA-5 | dsRNA | Type I IFN, pro-inflammatory cytokines, antibody, CD4/CD8 response |
| cGAMP, C-di-GMP | STING | Cyclic dinucleotide | Type I IFN, pro-inflammatory cytokines, antibody, CD8 response |

Based on Rappuoli R et al. Nat Rev Immunol 2011;11:865-72 [1].

TLR, Toll-like receptor; IFN, interferon.
Mucosal Vaccines and Delivery Routes

Mucosal vaccination can induce antigen-specific humoral and cell-mediated immune responses in both the systemic and mucosal compartments [14]. Additionally, such vaccination efficiently induces long-lasting B- and T-cell memory [15]. Importantly, the characteristics of mucosal immune response induction depend on the vaccine delivery route chosen (Fig. 3). For example, oral delivery (a traditional form of mucosal vaccination) can induce production of antigen-specific SlgA in the gastrointestinal tract, salivary glands, and mammary glands [14]. Currently, the licensed human live attenuated vaccines for rotavirus, poliovirus, Salmonella Typhi, and cholera are delivered orally (Table 3) [14,16]. Intranasal vaccines such as FluMist, a live attenuated influenza virus vaccine, generate SlgA in the upper and lower respiratory, gastric, and genital tracts [17]. Upon sublingual vaccination, antigen-specific immune responses are induced in the gastrointestinal and the upper and lower respiratory tracts [14].
Although a few human mucosal vaccines are licensed, safety issues remain; the current vaccines are live attenuated or non-living whole-cell vaccines (Table 3). Subunit vaccines lacking entire pathogens are considered to be safer next-generation vaccines. However, several issues must be addressed when developing subunit mucosal vaccines, including poor immunogenicity, degradation of vaccine materials in the harsh mucosal environment, delivery of vaccine materials to mucosal immune inductive tissue, and modulation of the mucosal immune environment such that oral tolerance does not develop.

| Pathogen          | Trade name                      | Composition                                      | Dosage         | Immunological mechanism                          | Efficacy                                |
|-------------------|--------------------------------|--------------------------------------------------|----------------|--------------------------------------------------|-----------------------------------------|
| Rotavirus         | Rotarix, RotaTeg               | Live attenuated, monovalent or pentavalent rotaviruses | Oral, 3 doses | Mucosal IgA and systemic neutralizing IgG         | Over 70%-90% against severe disease    |
| Poliovirus        | OPV, Poliomyelitis vaccine     | Live attenuated trivalent, bivalent, and monovalent polioviruses | Oral, 3 doses | Mucosal IgA and systemic IgG                     | Over 90% in most of the world          |
| Salmonella Typhi  | Vivotif, Ty21A                 | Live attenuated S. Typhi bacteria                | Oral, 3-4 doses | Mucosal IgA, systemic IgG, and CTL responses     | Variable, but more than 50%            |
| V. cholera        | Dukoral, ORC-Vax, Shanchol     | Inactivated V. cholera O1 classical and El Tor biotypes with or without CTB | Oral, 2-3 doses | Antibacterial, toxin-specific, and LPS-specific IgA | Strong herd protection over 85%        |
| Influenza type A  | FluMist                        | Live viral reassortant with trivalent mix of H1, H3, and B strains of hemagglutinin and neuraminidase genes in an attenuated donor strain | Intranasal in young children, 2 doses | Hemagglutinin- and neuraminidase-specific mucosal IgA and systemic IgG responses | >85% in children, variable in adults   |

Table 3. Currently licensed mucosal vaccines

Table 4. M cell–specific molecules and their ligands

| Ligand                        | Receptors on M cells | Reference |
|-------------------------------|----------------------|-----------|
| Ulex europaeus 1 (UEA-1)      | α1,2 fucose          | [21]      |
| Aleuria auranita (AAL)        | α-L-fucose           | [20]      |
| Galectin-9                    | N-glycans/repeated oligosaccharide | [22]      |
| Peptide Co1 (SFHQLPARSLPLP)   | C5aR                 | [23]      |
| Cathelicidin LL-37            | P2X7 receptor, Formyl peptide receptor 2 | [24] |
| Antibody NKM 16:2-4           | α1,2 fucose-containing carbohydrate | [33] |
| Antibody LM112                | Sialyl Lewis A       | [29]      |
| Antibody 367-H9               | Glycoprotein 2       | [27]      |
| α1 protein (reovirus)         | α2,3 sialic acid     | [39]      |
| Invasion (Yersinia)           | β1 integrin          | [26]      |
| Long polar fimbriae (Escherichia coli, Salmonella) | Unknown | [29] |
| FimH (E. coli, Salmonella)    | Glycoprotein 2/Uromodulin | [36] |
| OmpH (Yersinia)               | C5aR                 | [23]      |
| LPS                            | TLR-4                | [28]      |
| Lipoteichoic acid             | TLR-2                | [37]      |
| Phosphorylcholine moiety of LPS | PFAR                | [38]      |
| Hsp60 of Brucella abortus     | Cellular prion protein | [32] |
| Lipid A domain of LPS (gram-negative bacteria) | ANXA5 | [35] |
| Bacterial peptidoglycan       | PGLRP-1              | [34]      |
| SIgA                          | Unknown              | [30]      |
| c-term domain of enterotoxin (Clostridium perfringens) | Claudin 4 | [31] |

Adapted from Lycke N. Nat Rev Immunol 2012;12:592-605 [14] and Kim SH and Jang YS. Exp Mol Med 2014;46:e85 [16].

Adapted from Kunisawa J, et al. Adv Drug Deliv Rev 2012;64:523-30 [29] and Kim SH and Jang YS. Exp Mol Med 2014;46:e85 [16].

LPS, lipopolysaccharide; Hsp60, heat shock protein 60; SIgA, secretory IgA.
The mucosa is continuously exposed to various antigens and microbiota and tightly regulates the influx of luminal antigens. Therefore, special delivery systems are required for development of successful mucosal vaccines [18]. M cells are the ideal targets of mucosal vaccine materials. Not only are the cells localized to the FAE of mucosal immune inductive sites, but many APCs are located nearby and/or under pockets of M cells. Although antigen uptake by M cells was previously thought to be non-specific, many recent studies have shown that a specific antigen delivery mechanism is involved [19]. GP2, a protein expressed specifically by M cells, drives transcytosis of FimH$^+$ bacteria into such cells. Therefore, M cell-specific markers can be utilized for antigen delivery to mucosal immune inductive sites [20-39] (Table 4). For example, an M cell-specific antibody, NKM 16-2-4, recognizes the α(1,2)-fucose-containing carbohydrate moiety of M cells and can be used to enhance delivery of an associated antigen [33]. Additionally, an M cell-targeting ligand, Co1, also targets antigens to M cells by interacting with the complement 5a receptor, inducing an antigen-specific immune response [23,40]. Thus, M cell-targeting of vaccine materials will play a pivotal role in successful mucosal vaccination.

In the tolerogenic mucosal environment, adjuvants with immunostimulatory capacities enhance immune induction (Table 5) [37,41-49]. When TLR agonists such as Pam3CSK4, poly(I:C), MPL, or CpG-ODN were given either nasally or orally, in combination with vaccine materials, both systemic and mucosal antigen-specific immune responses were enhanced [3]. In addition, some immunostimulatory adjuvants improve the quality of the immune response. Cholera toxin (CT) is an effective mucosal vaccine adjuvant because it interacts with the GM1 ganglioside. However, the use of CT in this context raises a safety concern. Thus, CTA1-DD, which contains a mutant GM1 ganglioside-targeting A subunit of CT and the D-fragment of Staphylococcus aureus protein A to activate follicular dendritic cells (FDCs) closely associated with GCs, has been developed. CTA1-DD effectively promotes the induction of high-affinity B-cell clones and long-lived memory B cells and plasma cells [50]. Another mucosal vaccine adjuvant is the oil-in water emulsion MF59, which is currently licensed for human use. Although the mechanism of action remains unclear, MF59 not only enhances recruitment of innate immune cells via release of ATP and antigen uptake, but it also increases the adjunctive capacities of B cells by enhancing GC actions via activation of follicular helper T cells [51]. Finally, cathelicidin LL-37 is an immunostimulatory adjuvant that targets antigens to M cells. LL-37 increases antigen delivery to such cells and activates FDCs by interacting with the formyl peptide receptor 2 [24]. This enhances the induction of antigen-specific immune responses in both the systemic and mucosal compartments.

**Conclusion**

Recently, the need for mucosal vaccines has become recognized. Such vaccines offer several advantages including safe-
ty, convenience of vaccination, economical production, induction of mucosal immune responses, and enhanced memory B- and T-cell induction. However, several hurdles must be overcome in the development of practical subunit mucosal vaccines, including poor immunogenicity, degradation of vaccine materials in a harsh mucosal environment, delivery of vaccine materials to mucosal immune inductive tissue, and modulation of the mucosal immune environment to ensure that oral tolerance does not develop. These obstacles will be overcome by developing effective mucosal adjuvants that target M cells and are immunostimulatory.

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References

1. Rappuoli R, Mandl CW, Black S, De Gregorio E. Vaccines for the twenty-first century society. Nat Rev Immunol 2011;11:865-72.
2. Kwok R. Vaccines: the real issues in vaccine safety. Nature 2011;473:436-8.
3. Gutjahr A, Tiraby G, Perouzel E, Verrier B, Paul S. Triggering intracellular receptors for vaccine adjuvantation. Trends Immunol 2016;37:573-87.
4. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol 2010;10:787-96.
5. McGhee JR, Fujihashi K. Inside the mucosal immune system. PLoS Biol 2012;10:e1001397.
6. Brandtzaeg P, Kiyono H, Pabst R, Russell MW. Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunol 2008;1:31-7.
7. Corr SC, Gahan CC, Hill C. M-cells: origin, morphology and role in mucosal immunity and microbial pathogenesis. FEMS Immunol Med Microbiol 2008;52:2-12.
8. Ohno H. Intestinal M cells. J Biochem 2016;159:151-60.
9. Schulz O, Pabst O. Antigen sampling in the small intestine. Trends Immunol 2013;34:155-61.
10. Cerutti A, Chen K, Chorny A. Immunoglobulin responses at the mucosal interface. Annu Rev Immunol 2011;29:273-93.
11. Bemark M, Boysen P, Lycke NY. Induction of gut IgA production through T cell-dependent and T cell-independent pathways. Ann N Y Acad Sci 2012;1247:97-116.
12. Pabst O. New concepts in the generation and functions of IgA. Nat Rev Immunol 2012;12:821-32.
13. Strugnell RA, Wijburg OL. The role of secretory antibodies in infection immunity. Nat Rev Microbiol 2010;8:656-67.
14. Lycke N. Recent progress in mucosal vaccine development: potential and limitations. Nat Rev Immunol 2012;12:592-605.
15. Sallusto F, Lanzavecchia A, Araki K, Ahmed R. From vaccines to memory and back. Immunity 2010;33:451-63.
16. Kim SH, Jang YS. Antigen targeting to M cells for enhancing the efficacy of mucosal vaccines. Exp Mol Med 2014;46:e85.
17. Pedersen G, Cox R. The mucosal vaccine quandary: intranasal vs. sublingual immunization against influenza. Hum Vaccin Immunother 2012;8:689-93.
18. Lamichhane A, Azegamia T, Kiyono H. The mucosal immune system for vaccine development. Vaccine 2014;32:6711-23.
19. Mabott NA, Donaldson DS, Ohno H, Williams IR, Mahajan A. Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. Mucosal Immunol 2013;6:666-77.
20. Clark MA, Jepson MA, Simmons NL, Hirst BH. Differential surface characteristics of M cells from mouse intestinal Peyer’s and caecal patches. Histochem J 1994;26:271-80.
21. Foster N, Clark MA, Jepson MA, Hirst BH. Ulex europaeus 1 lectin targets microspheres to mouse Peyer’s patch M-cells in vivo. Vaccine 1998;16:536-41.
22. Hirabayashi J, Hashidate T, Arata Y, et al. Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. Biochim Biophys Acta 2002;1572:232-54.
23. Kim SH, Jung DJ, Yang IY, et al. M cells expressing the complement C5a receptor are efficient targets for mucosal vaccine delivery. Eur J Immunol 2011;41:3219-29.
24. Kim SH, Lee HY, Jang YS. Expression of the ATP-gated P2X7 receptor on M cells and its modulating role in the mucosal immune environment. Immune Netw 2015;15:44-9.
25. Kim SH, Yang IY, Kim J, Lee KY, Jang YS. Antimicrobial peptide LL-37 promotes antigen-specific immune responses in mice by enhancing Th17-skewed mucosal and systemic immunities. Eur J Immunol 2015;45:1402-13.
26. Clark MA, Hirst BH, Jepson MA. M-cell surface beta1 integrin expression and invasin-mediated targeting of Yersinia pseudotuberculosis to mouse Peyer’s patch M cells. Infect Immun 1998;66:1237-43.
27. Hase K, Kawano K, Nochi T, et al. Uptake through glyco-
protein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. Nature 2009;462:226-30.

28. Keely S, Glover LE, Weissmueller T, et al. Hypoxia-inducible factor-dependent regulation of platelet-activating factor receptor as a route for gram-positive bacterial translocation across epithelia. Mol Biol Cell 2010;21:538-46.

29. Kunisawa J, Kurashima Y, Kiyono H. Gut-associated lymphoid tissues for the development of oral vaccines. Adv Drug Deliv Rev 2012;64:523-30.

30. Kyd JM, Cripps AW. Functional differences between M cells and enterocytes in sampling luminal antigens. Vaccine 2008;26:6221-4.

31. Lo DD, Ling J, Eckelhoefer AH. M cell targeting by a Claudin 4 targeting peptide can enhance mucosal IgA responses. BMC Biotechnol 2012;12:7.

32. Nakato G, Hase K, Suzuki M, et al. Cutting Edge: Brucella abortus exploits a cellular prion protein on intestinal M cells as an invasive receptor. J Immunol 2012;189:1540-4.

33. Nochi T, Yuki Y, Matsumura A, et al. A novel M cell-specific carbohydrate-targeted mucosal vaccine effectively induces antigen-specific immune responses. J Exp Med 2007;204:2789-96.

34. Osanai A, Sashinami H, Asano K, Li SJ, Hu DL, Nakane A. Mouse peptidoglycan recognition protein PGLYRP-1 plays a role in the host innate immune response against Listeria monocytogenes infection. Infect Immun 2011;79:858-66.

35. Rand JH, Wu XX, Lin EY, Griffel A, Gialanella P, McKitrick JC. Annexin A5 binds to lipopolysaccharide and reduces its endotoxin activity. MBio 2012;3:e00292-11.

36. Sato S, Kaneto S, Shibata N, et al. Transcription factor Spi-B-dependent and -independent pathways for the development of Peyer’s patch M cells. Mucosal Immunol 2013;6:838-46.

37. Shafique M, Wilschut J, de Haan A. Induction of mucosal and systemic immunity against respiratory syncytial virus by inactivated virus supplemented with TLR9 and NOD2 ligands. Vaccine 2012;30:597-606.

38. Tyrer P, Foxwell AR, Cripps AW, Apicella MA, Kyd JM. Microbial pattern recognition receptors mediate M-cell uptake of a gram-negative bacterium. Infect Immun 2006;74:625-31.

39. Wolf JL, Kauffman RS, Finberg R, Dambrauskas R, Fields BN, Trier JS. Determinants of reovirus interaction with the intestinal M cells and absorptive cells of murine intestine. Gastroenterology 1983;85:291-300.

40. Kim SH, Seo KW, Kim J, Lee KY, Jang YS. The M cell-targeting ligand promotes antigen delivery and induces antigen-specific immune responses in mucosal vaccination. J Immunol 2010;185:5787-95.

41. Agren LC, Ekman L, Lowenadler B, Lycke NY. Genetically engineered nontoxic vaccine adjuvant that combines B cell targeting with immunomodulation by cholera toxin A1 subunit. J Immunol 1997;158:3936-46.

42. Chen K, Cerutti A. Vaccination strategies to promote mucosal antibody responses. Immunity 2010;33:479-91.

43. Christensen D, Agger EM, Andreasen LV, Kirby D, Andersen P, Perrie Y. Liposome-based cationic adjuvant formulations (CAF): past, present, and future. J Liposome Res 2009;19:2-11.

44. Eliasson DG, Helgeby A, Schon K, et al. A novel non-toxic combined CTA1-DD and ISCOMS adjuvant vector for effective mucosal immunization against influenza virus. Vaccine 2011;29:3951-61.

45. Manicassamy S, Pulendran B. Modulation of adaptive immunity with Toll-like receptors. Semin Immunol 2009;21:185-93.

46. Thompson AL, Johnson BT, Sempowski GD, et al. Maximal adjuvant activity of nasally delivered IL-1alpha requires adjuvant-responsive CD11c(+) cells and does not correlate with adjuvant-induced in vivo cytokine production. J Immunol 2012;188:2834-46.

47. Uematsu S, Fujimoto K, Jang MH, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat Immunol 2008;9:769-76.

48. van der Lubben IM, Verhoef JC, Borchard G, Junginger HE. Chitosan for mucosal vaccination. Adv Drug Deliv Rev 2001;52:139-44.

49. Winstone N, Wilson AJ, Morrow G, et al. Enhanced control of pathogenic Simian immunodeficiency virus SIV-mac239 replication in macaques immunized with an interleukin-12 plasmid and a DNA prime-viral vector boost vaccine regimen. J Virol 2011;85:9578-87.

50. Agren L, Sverremark E, Ekman L, et al. The ADP-ribosylating CTA1-DD adjuvant enhances T cell-dependent and independent responses by direct action on B cells involving anti-apoptotic Bcl-2- and germinal center-promoting effects. J Immunol 2000;164:6276-86.

51. Mastelic Gavillet B, Eberhardt CS, Auderset F, et al. MF59 Mediates Its B Cell Adjuvanticity by Promoting T Follicular Helper Cells and Thus Germinal Center Responses in Adult and Early Life. J Immunol 2015;194:4836-45.