

macrophages warrants analysis. Moseman et al. (2012) go on to show that B cell-derived LT α 1 β 2 is critical for inducing the protective phenotype of the SCS macrophages. When B cells are the only cell type that cannot produce LT α 1 β 2, macrophages are able to capture lymph-borne viral particles but the virus does not replicate and type I IFN secretion is compromised. An outstanding question is what factors allow virus replication to proceed—can the effects all be attributed to the inability of SCS macrophages to respond to autocrine as well as perhaps paracrine type I interferon? The study by Moseman et al. (2012) along with other available data suggest the fascinating possibility that the immune system has evolved to allow a certain proscribed degree of microbial growth to “prime the pump” in order to promote both rapid innate immunity and provide sufficient antigenic material and inflammatory signals to promote adaptive immunity to

prevent entry of pathogens upon secondary encounter. In fact, recent findings indicate that a specialized subset of dendritic cells are permissive for the initial replication of *Listeria monocytogenes* that leads to the induction of a rapid innate response leading to the generation of a protective T cell response (Edelson et al., 2011). Additional research will be needed to determine whether events such as these are more commonplace than previously appreciated.

REFERENCES

Benedict, C.A., Banks, T.A., Senderowicz, L., Ko, M., Britt, W.J., Angulo, A., Ghazal, P., and Ware, C.F. (2001). *Immunity* 15, 617–626.

Ciavarra, R.P., Taylor, L., Greene, A.R., Yousefieh, N., Horeth, D., van Rooijen, N., Steel, C., Gregory, B., Birkenbach, M., and Sekellick, M. (2005). *Virology* 342, 177–189.

Delale, T., Paquin, A., Asselin-Paturel, C., Dalod, M., Brizard, G., Bates, E.E., Kastner, P., Chan, S.,

Akira, S., Vicari, A., et al. (2005). *J. Immunol.* 175, 6723–6732.

Edelson, B.T., Bradstreet, T.R., Hildner, K., Carrero, J.A., Frederick, K.E., Kc, W., Belizaire, R., Aoshi, T., Schreiber, R.D., Miller, M.J., et al. (2011). *Immunity* 35, 236–248.

García-Sastre, A., and Biron, C.A. (2006). *Science* 312, 879–882.

Honke, N., Shaabani, N., Cadeddu, G., Sorg, U.R., Zhang, D.E., Trilling, M., Klingel, K., Sauter, M., Kandolf, R., Gailus, N., et al. (2012). *Nat. Immunol.* 13, 51–57.

Iannacone, M., Moseman, E.A., Tonti, E., Bosurgi, L., Junt, T., Henrickson, S.E., Whelan, S.P., Guidotti, L.G., and von Andrian, U.H. (2010). *Nature* 465, 1079–1083.

Mebius, R.E., Nolte, M.A., and Kraal, G. (2004). *Crit. Rev. Immunol.* 24, 449–464.

Moseman, E.A., Iannacone, M., Bosurgi, L., Tonti, E., Chevrier, N., Tumanov, A., Fu, Y.-X., Hacohen, N., and von Andrian, U.H. (2012). *Immunity* 36, this issue, 415–426.

Schneider, K., Loewendorf, A., De Trez, C., Fulton, J., Rhode, A., Shumway, H., Ha, S., Patterson, G., Pfeffer, K., Nedospasov, S.A., et al. (2008). *Cell Host Microbe* 3, 67–76.

Natural “Helper” Cells in the Lung: Good or Bad Help?

Shigeo Koyasu^{1,2,3,*} and Kazuyo Moro^{1,2,4}

¹Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo 160-8582, Japan

²Laboratory for Immune Cell System, RIKEN Research Center for Allergy and Immunology (RCI), Kanagawa 230-0045, Japan

³Research Center for Science Systems, Japan Society for the Promotion of Science (JSPS), Tokyo 102-8472, Japan

⁴Japan Science and Technology Agency (JST), Precursory Research for Embryonic Science and Technology (PRESTO), Tokyo 102-0076, Japan

*Correspondence: koyasu@z3.keio.jp

DOI 10.1016/j.immuni.2012.03.001

The natural helper (NH) cell comprises a newly identified Th2 cell-type innate lymphocyte population. In this issue of *Immunity*, Halim et al. (2012) provide evidence that NH cells reside in the lung and play a critical role in protease allergen-induced airway inflammation.

Proteases are important components of many allergens (Reed and Kita, 2004). For example, papain is a well-known protease that causes occupational asthma, and native papain but not heat-inactivated papain induces lung inflammation in mice. House dust mites also produce a well-known allergen whose allergy-inducing ability is largely dependent on its protease activity. In addition, invasion of host tissues by helminthes

occurs via high protease activities and helminth infection induces rapid activation of Th2 cell-type inflammatory responses. Proteases are thought to disrupt mucosal integrity by digesting cell adhesion molecules and further act on protease-activated receptors to activate airway epithelial cells. The cytokines thymic stroma lymphopoietin (TSLP), IL-25, and IL-33 derived from epithelial cells activated in this way induce rapid

Th2 cell-type inflammation. Polymorphisms of IL-33 and its receptor ST2 are associated with allergic diseases, including asthma in humans, demonstrating the importance of epithelial cell-derived cytokines for the onset of Th2 cell-type immune responses.

Papain causes asthma-like symptoms associated with Th2 cell-type reactions such as eosinophilia and goblet cell hyperplasia in Rag-deficient mice. These

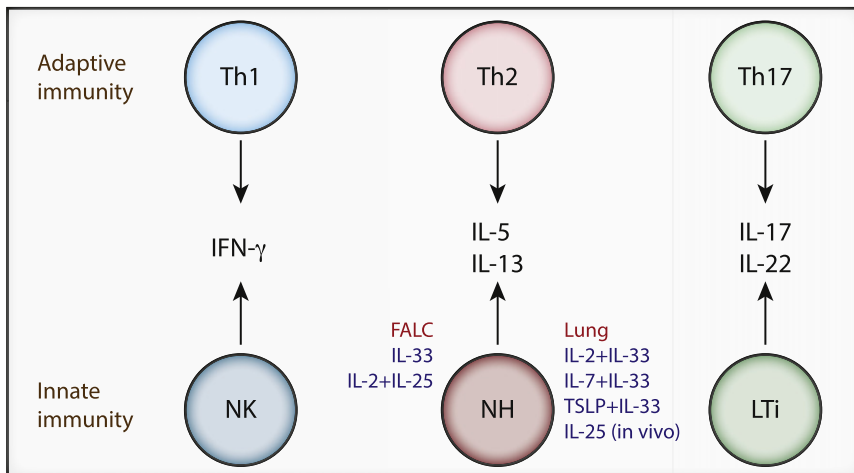


Figure 1. Distinct Types of "Helper" Cells in Innate and Adaptive Immunity

NK cells, Th2 cell-type innate lymphocytes such as NH cells, and $\text{ROR}\gamma^+$ LTI-related cells seem to play distinct roles in innate immune responses by producing Th1, Th2, and Th17 cell-type cytokines, respectively. Such subsets correspond to Th1, Th2, and Th17 cells in adaptive immunity. Both FALC NH cells and lung NH cells produce large amounts of IL-5 and IL-13 but not IL-4. However, the cytokine requirements for the production of IL-5 and IL-13 are different between FALC NH cells and lung NH cells in that lung NH cells require costimulation by IL-2, IL-7, or TSLP in order to respond to IL-33. Isolated lung NH cells do not respond to IL-25 but IL-25 is able to activate lung NH cells *in vivo*, indicating the presence of a costimulatory cytokine(s) in the lung tissue. NH cells are observed in various tissues including bone marrow and liver in addition to mesentery and lung.

mice lack T and B cells, so this indicates the involvement of non-T and non-B cells in the process (Oboki et al., 2010). Similarly, helminth infection of Rag-deficient mice results in rapid induction of IL-5 and IL-13, indicating a role for non-T and non-B cells in helminth-induced rapid Th2 cell-related reactions. In 2010, an innate lymphocyte subset was identified producing Th2 cell-type cytokines, most notably IL-5 and IL-13, and variously named natural helper (NH) cells, MPP type 2, nuocytes, or innate helper type 2 (Ih2) (Moro et al., 2010; Saenz et al., 2010). NH cells are present in the fat-associated lymphoid cluster (FALC) of naive mice (Moro et al., 2010). MPP type 2, nuocytes, and Ih2 were observed in the spleen and lymph nodes of IL-4-GFP or IL-13-GFP reporter mice upon helminth infection or administration of IL-25 or IL-33 (Saenz et al., 2010).

In this issue of *Immunity*, Halim et al. (2012) demonstrate that NH cells residing in the lung of naive mice play a critical role in papain-induced rapid Th2 cell-type inflammation. The authors named these cells lung NH cells or LNH cells. LNH cells are defined as $\text{Lin}^- \text{c-Kit}^{+/\text{lo}} \text{Sca-1}^+ \text{CD25}^+ \text{CD127}^+$ cells and are quite similar to NH cells in the FALC. Like FALC NH cells, LNH cells are present in Rag-defi-

cient mice but absent in γc -deficient mice. Purified LNH cells produce IL-5 and IL-13 in response to a combination of phorbol myristate acetate (PMA) and ionomycin. Low amounts of IL-4, IL-17A, and IFN- γ were also produced upon PMA and ionomycin stimulation but, like FALC NH cells, LNH cells produce IL-3, IL-4, IL-17A, and IFN- γ only in response to PMA and ionomycin. Unlike FALC NH cells, LNH cells do not produce IL-6. In addition, whereas isolated FALC NH cells constitutively produce IL-5, IL-6, and IL-13, isolated naive LNH cells do not produce detectable amounts of IL-5 protein. Another significant difference between LNH cells and FALC NH cells is that IL-33 alone is able to activate FALC NH cells but not LNH cells (Figure 1). Costimulation with other cytokines such as IL-2, IL-7, or TSLP is required for the induction of large amounts of IL-5 and IL-13 from LNH cells. Such a requirement for costimulation was also observed for nuocytes (Neill et al., 2010). Although LNH cells express IL-25 receptors, IL-25 had little effect on cytokine production by isolated LNH cells.

Intranasal administration of papain induced the production of IL-5 and IL-13 in the lung and caused lung inflammation associated with eosinophilia and goblet

cell hyperplasia in wild-type and Rag-deficient mice but not in Rag- γc double-deficient mice. Administration of CD25 mAb in Rag-deficient mice resulted in the depletion of LNH cells and a significant decrease in papain-induced lung inflammation. Adoptive transfer of isolated LNH cells into Rag- γc double-deficient mice restored papain-induced lung inflammation, collectively demonstrating the role of LNH cells in papain-induced lung inflammation. However, it is possible that NH cells in other organs and tissues such as FALC are recruited to the lung upon papain administration. In order to examine whether LNH cells residing in the lung are sufficient to produce papain-induced lung inflammation, Halim et al. (2012) applied various stimuli to lung explants. Interestingly, production of IL-5 and IL-13 was readily observed in lung explant cultures upon stimulation by IL-25 alone, although IL-25 was unable to stimulate purified LNH cells *in vitro*. IL-5 was produced by lung explants in the absence of any stimulation and a combination of IL-2 and IL-7 induced IL-5 and IL-13 from lung explants in contrast to their activity in *in vitro* cultures of LNH cells. As expected, neither IL-5 nor IL-13 was produced from lung explants of Rag- γc double-deficient mice. Thus a clear gap exists between cytokine production by isolated LNH cells compared to lung explants. A likely explanation is that papain acts on lung tissue to induce soluble factors that stimulate LNH cells. Indeed, production of TSLP, IL-25, and IL-33 was readily observed in lung explant cultures and papain treatment enhanced the production of these cytokines. Neutralization of TSLP or IL-33 suppressed the production of IL-5 and IL-13 induced by papain or IL-25, demonstrating the importance of TSLP and IL-33.

Questions remain. It is still unclear how LNH cells are activated upon papain administration. Purified LNH cells are unable to produce IL-5 or IL-13 in response to IL-25 even with IL-2 and IL-7, yet IL-25 alone is sufficient to induce IL-5 and IL-13 in lung explant cultures. What acts on LNH cells synergistically with IL-25? Or, what is induced by IL-25? Does IL-25 induce TSLP and IL-33 from lung tissue? It is intriguing that significant amounts of IL-5, IL-13, and IL-33 are continuously produced

by lung explants in a time-dependent manner without papain. If this were the case in vivo, one would expect spontaneous eosinophilia in the lung. One caveat of the lung explant cultures is that lung tissues were sliced into pieces before culture. IL-33 is localized in the nucleus and inactivated by caspases 3 and 7 during apoptosis but full-length IL-33 is released from cells upon necrotic death and functions as an alarmin to stimulate a variety of cells. Although IL-33 protein is constitutively expressed in the lung (Oboki et al., 2010), it is unlikely that active IL-33 is released from the tissue. However, slicing lung tissue is likely to cause necrotic cell death and induce the release of IL-33. It is also possible that such treatment alters the cytokine production in the lung explant culture system by other, undefined mechanisms.

Other groups have also reported the presence in the lung of NH cells that respond to influenza virus infection (Chang et al., 2011; Monticelli et al., 2011). Chang et al. (2011) demonstrated that NH cells in the lung are involved in the airway hypersensitivity induced upon infection by H3N1 influenza virus. The NH cells studied by Chang et al. (2011) produced greatly reduced amounts of Th2 cell-type cytokines compared to the LNH cells reported by Halim et al. (2012). It is possible that Chang et al. (2011) used BALB/c mice whereas other groups, including Halim et al. (2012), used C57BL/6 mice. Alternatively, differences in environmental cues, such as interferons produced upon viral infection, could affect the functions of NH cells. Monticelli et al. (2011) also reported that NH cells in the lung are involved in the tissue inflam-

mation caused by infection of influenza virus of the H1N1 strain. They further demonstrated that amphiregulin is produced by NH cells and plays an important role in the maintenance of epithelial integrity in the airway. Wilhelm et al. (2011) reported that innate lymphocytes in the lung produce IL-9 and that such IL-9 production is involved in the production of IL-5 and IL-13. For now it is unclear whether the IL-9 producer in the lung is the NH cell or a distinct type of innate lymphocyte population but further investigation should clarify the functional relationship between IL-9 and NH cells in the lung.

It is now evident that NH cells are present in many organs and tissues including mesenteric FALC, lung, bone marrow, and liver (Moro et al., 2010; Chang et al., 2011; Monticelli et al., 2011; Halim et al., 2012). NH cells are important in the innate immune response against helminth infection in the gut (Moro et al., 2010) and probably play a role during the lung stage of helminth infection as well (Yasuda et al., 2012). It will be of interest to determine whether NH cells in different organs and tissues are involved in various inflammatory responses in distinct organs. Recent studies have identified multiple subsets of innate lymphocytes, which may correspond to Th cell subsets in adaptive immune responses. NK cells, LTi cells, and Th2 cell-type innate lymphocytes such as NH cells play distinct roles in innate immune responses by producing Th1, Th17, and Th2 cell-type cytokines, respectively (Figure 1). Cooperation between these innate lymphocytes and antigen-specific T and B cells are probably important in the pathophysiology of

various inflammatory responses including protective immunity against various microbes and allergic diseases such as asthma.

ACKNOWLEDGMENTS

S.K. is a consultant for Medical and Biological Laboratories, Co. Ltd.

REFERENCES

- Chang, Y.J., Kim, H.Y., Albacker, L.A., Baumgarth, N., McKenzie, A.N., Smith, D.E., Dekruyff, R.H., and Umetsu, D.T. (2011). *Nat. Immunol.* 12, 631–638.
- Halim, T.Y.F., Krauß, R.H., Sun, A.C., and Takei, F. (2012). *Immunity* 36, this issue, 451–463.
- Monticelli, L.A., Sonnenberg, G.F., Abt, M.C., Alenqhat, T., Ziegler, C.G., Doering, T.A., Angelosanto, J.M., Laidlaw, B.J., Yang, C.Y., Sathaliyawala, T., et al. (2011). *Nat. Immunol.* 12, 1045–1054.
- Moro, K., Yamada, T., Tanabe, M., Takeuchi, T., Ikawa, T., Kawamoto, H., Furusawa, J., Ohtani, M., Fujii, H., and Koyasu, S. (2010). *Nature* 463, 540–544.
- Neill, D.R., Wong, S.H., Bellosi, A., Flynn, R.J., Daly, M., Langford, T.K., Bucks, C., Kane, C.M., Fallon, P.G., Pannell, R., et al. (2010). *Nature* 464, 1367–1370.
- Oboki, K., Ohno, T., Kajiwara, N., Arae, K., Morita, H., Ishii, A., Nambu, A., Abe, T., Kiyonari, H., Matsumoto, K., et al. (2010). *Proc. Natl. Acad. Sci. USA* 107, 18581–18586.
- Reed, C.E., and Kita, H. (2004). *J. Allergy Clin. Immunol.* 114, 997–1008, quiz 1009.
- Saenz, S.A., Noti, M., and Artis, D. (2010). *Trends Immunol.* 31, 407–413.
- Wilhelm, C., Hirota, K., Stieglitz, B., Van Snick, J., Tolaini, M., Lahl, K., Sparwasser, T., Helmby, H., and Stockinger, B. (2011). *Nat. Immunol.* 12, 1071–1077.
- Yasuda, K., Muto, T., Kawagoe, T., Matsumoto, M., Sasaki, Y., Matsushita, K., Taki, Y., Futatsugi-Yumikura, S., Tsutsui, H., Ishii, K.J., et al. (2012). *Proc. Natl. Acad. Sci. USA* 109, 3451–3456.