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Development of T_H1 $CD4^+$ T Cells Through IL-12 Produced by *Listeria*-Induced Macrophages

Chyi-Song Hsieh, Steven E. Macatonia, Catherine S. Tripp, Stanley F. Wolf, Anne O'Garra, Kenneth M. Murphy*

Development of the appropriate $CD4^+$ T helper (T_H) subset during an immune response is important for disease resolution. With the use of naïve, ovalbumin-specific αβ T cell receptor transgenic T cells, it was found that heat-killed *Listeria monocytogenes* induced T_H1 development in vitro through macrophage production of interleukin-12 (IL-12). Moreover, inhibition of macrophage production of IL-12 may explain the ability of IL-10 to suppress T_H1 development. Murine immune responses to *L. monocytogenes* in vivo are of the appropriate T_H1 phenotype. Therefore, this regulatory pathway may have evolved to enable innate immune cells, through interactions with microbial pathogens, to direct development of specific immunity toward the appropriate T_H phenotype.

Cytokine production by subsets of $CD4^+$ T cells regulates the effector responses to infectious organisms (*1*). The T_H1 subset produces IL-2, interferon-γ (IFN-γ), and lymphotoxin, facilitating cell-mediated immunity appropriate for intracellular and viral pathogens. The T_H2 subset produces IL-4, IL-5, and IL-6, which favors humoral immunity. Development of the appropriate T_H subset during an immune response is important because certain pathogens are most effectively controlled by either a predominantly T_H1- or T_H2-type immune response (*2, 3*). Because the host interacts with pathogens before the development of specific immunity, the directed development of the T_H phenotype may involve innate immune cells such as macrophages, natural killer (NK) cells, or mast cells (*4*). Thus, we examined whether discrete mechanisms regulate T_H phenotype development by host interactions with pathogens.

We employed an in vitro system using αβ T cell receptor (TCR) transgenic mice in which the majority of T cells are $CD4^+$, ovalbumin (OVA)-specific, $I\text{-}A^d$–restricted, and phenotypically naïve (*5, 6*). Thus, antigen-dependent naïve T cell activation and short-term differentiation can be studied in vitro without the use of polyclonal activators or long-term culture techniques (*6*). This system allows for experimental control over the antigen-presenting cell (APC), cytokine environment, and exposure to pathogens during primary T cell activation. Previously, it was found that both IL-4 (*6–8*) and IL-10 (*6*) inhibited T_H1 development in this system. IL-4 acted directly on T cells to promote T_H2 development, whereas IL-10 acted indirectly by suppressing the capacity of certain accessory cells to promote T_H1 development (*6, 9*). However, the relation of these and other in vitro, cytokine-mediated effects (*10*) to the physiologic regulation of T_H phenotype development by host interactions with pathogens was unclear.

Here, we present an explanation for the ability of a host to direct the T_H phenotype of a developing $CD4^+$ T cell response against a pathogen. *Listeria monocytogenes*, an intracellular Gram-positive bacterium that induces strong cellular immunity in vivo (*11*), also promotes the in vitro development of the T_H1 phenotype in OVA-specific $CD4^+$ transgenic T cells (Fig. 1) (*12*). In this report, we show that the mechanism of *Listeria*-induced T_H1 development involves an interaction between *Listeria* and host macrophages that results in IL-12 production. IL-12, then, directly induces T_H1 development in naïve $CD4^+$ T cells undergoing primary activation.

The addition of heat-killed *L. monocytogenes* (HKLM) during primary in vitro activation of OVA-specific T cells resulted in enhanced IFN-γ secretion in primary cultures and rapid development of the T_H1 phenotype (Fig. 1) (*12*). The effects of *Listeria* on T cells were not direct but were accessory cell–dependent (*12, 13*). When the B cell hybridoma TA3 (*6*) was the exclusive APC used for activation of transgenic T cells by OVA, the addition of *Listeria* had no effect either on IFN-γ production during the primary activation or on subsequent T_H phenotype development (Fig. 1, A and D) (*12*). By using TA3 as the exclusive APC to present antigen, we could test whether other cells added to TA3-stimulated cultures were able to mediate the effects of *Listeria* on the T_H phenotype independently of their ability to present antigen (*12, 13*). This was necessary because many cell types, including macrophages and resting B cells, are poor activators of naïve T cell proliferation (*6, 13, 14*), so that their use as APCs results in insufficient T cell recoveries and prevents evaluation of the T_H phenotype.

The addition of macrophages purified by cell sorting from $H\text{-}2^k$ (a nonpresenting haplotype) spleen to TA3-primed T cell cultures reconstituted the effects of *Listeria* observed in BALB/c ($H\text{-}2^d$) spleen-primed T cell cultures (Fig. 1, B to D; Fig. 2, E and F) (*12*). Full restoration of the effect occurred with 5×10^4 macrophages in which the *Listeria*-induced T_H1 phenotype was as pronounced as that produced by addition of

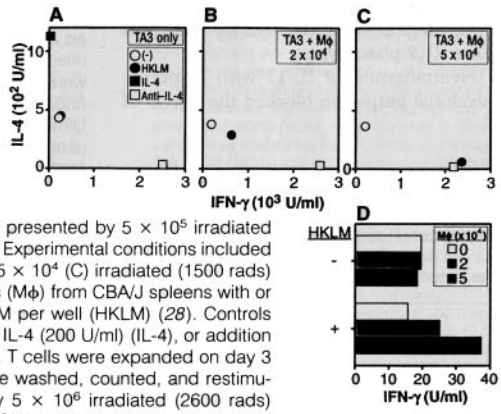

Fig. 1. Cellular requirements for the effects of *Listeria*. (**A** through **C**) Effects of HKLM on T_H phenotype development. T cell cultures were maintained as described (*12*). Briefly, $CD4^+$ T cells (2.5×10^5 from unimmunized transgenic mice) sorted by fluorescence-activated cell sorting (FACS) were activated under the indicated conditions with 0.3 μM OVA-peptide presented by 5×10^5 irradiated (10,000 rads) TA3 B hybridoma cells. Experimental conditions included the addition of 0 (A), 2×10^4 (B), or 5×10^4 (C) irradiated (1500 rads) bystander FACS-sorted Mac-1^+ cells (Mϕ) from CBA/J spleens with or without the addition of 2×10^7 HKLM per well (HKLM) (*28*). Controls included no addition (−), addition of IL-4 (200 U/ml) (IL-4), or addition of 11B11 mAb (10 μg/ml) (anti–IL-4). T cells were expanded on day 3 and harvested on day 7. T cells were washed, counted, and restimulated at 2.5×10^5 cells per well by 5×10^6 irradiated (2600 rads) BALB/c splenocytes with 0.3 μM OVA-peptide in the absence of experimental conditions to assess the effects of the indicated condition on T_H phenotype development. Supernatants were harvested after 48 hours and assayed for IL-4 and IFN-γ by enzyme-linked immunosorbent assay (ELISA) (*6*). (**D**) Effects of HKLM on IFN-γ production from naïve T cells. Supernatants were harvested on day 2 of the primary stimulation and analyzed for IFN-γ production by ELISA (*6*). The experiment was done three times with similar results. The reagents used and the method of isolation of T cells and macrophages have been described (*12*). Maximal effects in this system were consistently achieved with 5×10^4 macrophages.

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the monoclonal antibody (mAb) 11B11 (anti–IL-4) (Fig. 1C) (*6, 8*). Importantly, this restoration requires macrophage activation because addition of macrophages without *Listeria* had no effect either on IFN-γ production in primary cultures or on T_H phenotype development (Fig. 1). Other cell types, such as B cells, NK cells, and dendritic cells, are poor mediators of the effects of *Listeria* on T_H1 phenotype development (*13*). Thus, the macrophage is a critical cell for mediating the induction by *Listeria* of the T_H1 phenotype.

Macrophages could regulate the T_H phenotype either through direct cell-cell interactions or through soluble mediators. The ability of *H-2*^{k} haplotype macrophages to mediate the induction by *Listeria* of T_H1 development in OVA-specific, I-A^d–restricted T cells indicates that the effects of *Listeria* do not require direct major histocompatibility complex–TCR interactions. Additionally, *Listeria*-treated macrophages can promote T_H1 development through a semipermeable membrane, which suggests that a soluble factor mediates the effects of *Listeria* (*12*).

Interleukin-1, IL-6, tumor necrosis factor (TNF)–α, and transforming growth factor–β did not reproduce the induction by *Listeria* of the T_H1 phenotype (*12, 15*). The addition of IL-12 (*16–18*), however, skewed development of naïve CD4$^+$ transgenic T cells activated by either TA3 cells or BALB/c splenocytes to the T_H1 phenotype (Fig. 2, B and D) and augmented primary IFN-γ production (*18*) (Fig. 2, A and C). The effects of IL-12 were comparable to the effects of *Listeria* when splenic APCs were used (Fig. 2, C and D). However, when TA3 was the exclusive APC used, only IL-12 could induce T_H1 development (Fig. 1, A and D; Fig. 2, A and B). Thus, IL-12 alone is sufficient for induction of the T_H1 phenotype.

Neutralization of IL-12 with a specific polyclonal antiserum blocked the effects of *Listeria* on both IFN-γ production during the primary stimulation (Fig. 2E) and T_H1 phenotype development in the presence of splenic APCs (Fig. 2F). In contrast, neutralization of several other macrophage-derived cytokines had no effect on *Listeria*-induced T_H1 development (*12, 19*). Murine peritoneal macrophages (*20*) and human monocytes (*16*) can produce IL-12 in response to bacterial products, including *Listeria* (*20*). Thus, *Listeria*-induced T_H1 development appears to be the result of the production of IL-12 by macrophages.

Interleukin-10 inhibits T_H1 cytokine synthesis (*9, 21*) and T_H1 development in vitro (*6, 12*) and may suppress T_H1 responses in vivo (*22*). Interestingly, addition of IL-10 inhibited in vitro T_H1 development induced by *Listeria* (Fig. 2, E and F) (*12*) but not by IL-12 (Fig. 3). Because the effects of IL-10 are APC-dependent (*6, 9, 23*), these data suggest that IL-10 inhibits *Listeria*-induced IFN-γ production (*9, 21*) and T_H1 phenotype development (*12*) by blocking IL-12 production from macrophages.

Interleukin-12 increased primary IFN-γ production during naïve T cell activation (Table 1). However, IL-12 did not enhance IL-2 production (Table 1), which suggests that the effects of IFN-γ production did not result simply from enhanced T cell activation. Also, both the addition of IL-10 and the neutralization on IL-12 inhibited the induction by *Listeria* of IFN-γ production, but neither condition altered IL-2 production (*24*). Thus, IL-2 and IFN-γ expression in CD4$^+$ T cells involve separate activation pathways (*23*). Because IL-12 selectively enhances expression of IFN-γ during T cell activation, IL-12 might be considered an "IFN-γ costimulator."

Moreover, because of this enhancement of IFN-γ production during primary T cell activation, IFN-γ may be the final mediator of T_H1 development (*16, 25*). Although we have found the presence of IFN-γ to be necessary for *Listeria*-induced T_H1 development, the addition of IFN-γ to primary T cell cultures was not sufficient for T_H1 development (*12*). Also, IL-12 could act by simply inhibiting endogenous IL-4 production (*6, 8*). Although neutralization of IL-4

Table 1. IL-2 production during the primary stimulation with either TA3 or BALB/c APCs, assessed from the data shown in Fig. 2. Experimental conditions are as described in Fig. 2. ND, not done. Production of IFN-γ or IL-2 (U/ml) was determined by ELISA (*6*).

Conditions	TA3		BALB/c	
	IL-2	IFN-γ	IL-2	IFN-γ
(−)	283	20	429	85
Anti–IL-4	252	12	353	65
IL-4	252	20	377	92
HKLM	ND		242	301
IL-12 (10 U/ml)	261	97	303	423
IL-12 (1 U/ml)	260	86	297	348
IL-12 (0.1 U/ml)	289	73	327	234
IL-12 (10 U/ml)*	ND		<1	5

*No antigen added.

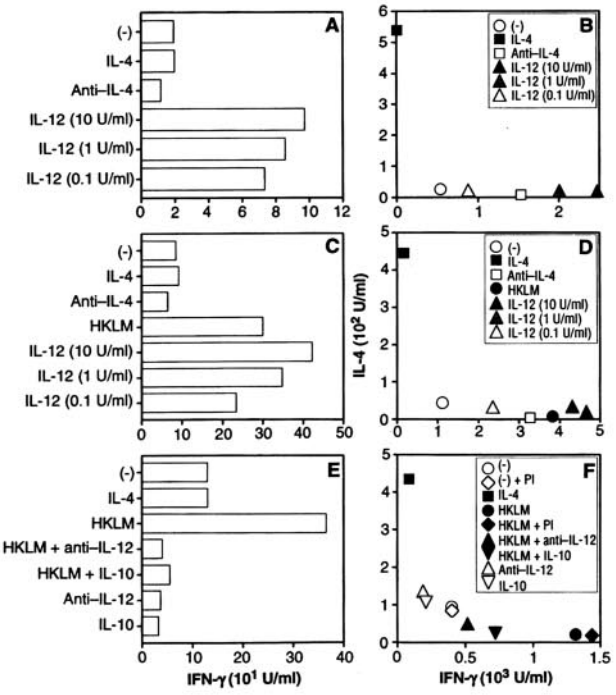

Fig. 2. The effects of IL-12 on naïve T cell responses and phenotype development. (**A** and **B**) Effects of IL-12 when only TA3 B hybridoma cells are the initiating APC. Nylon wool–purified T cells (2.5 × 10^5) (*12*) from unimmunized transgenic mice were stimulated with 5 × 10^5 irradiated (10,000 rads) TA3 B hybridoma cells and 0.3 μM OVA-peptide in the presence of the indicated concentrations of purified IL-12. Controls were no additions (−), addition of IL-4 (200 U/ml) (IL-4), and addition of mAb 11B11 (10 μg/ml) (anti–IL-4). Supernatants were harvested after 48 hours, and primary IFN-γ production (A) was assessed by ELISA (*6*). T cells were cultured as described in Fig. 1 and restimulated on day 7 with irradiated BALB/c splenocytes without the addition of experimental conditions. The T_H phenotype (B) was determined by ELISA analysis of the amounts of IL-4 and IFN-γ in the 48-hour supernatants (*6*). (**C** and **D**) Effects of IL-12 when BALB/c splenocytes are the initiating APC. T cells were cultured as above, except 5 × 10^6 irradiated (2600 rads) BALB/c splenocytes were used as the initiating APCs. The addition of 2 × 10^7 HKLM (HKLM) was used as a control for induction of the T_H1 phenotype. (**E** and **F**) Effects of anti–IL-12 on *Listeria*-induced T_H1 responses. T cells were cultured with BALB/c splenocytes as APCs as described in (C) and (D). Experimental conditions included the addition of rabbit anti–IL-12 serum at 1:1250 dilution (anti–IL-12) or IL-10 (50 U/ml) (IL-10) with or without addition of 2 × 10^7 HKLM (HKLM). Controls included no additions (−), addition of IL-4 (200 U/ml) (IL-4), or addition of preimmune serum at 1:1250 dilution (PI). These experiments were repeated at least three times with similar results.

did not enhance IFN-γ production during primary T cell activation, the subsequent effects of IL-12 on T_H phenotype development could involve reduction of the amount of IL-4. Therefore, IL-12 appears to act directly on T cells to promote T_H1 development, but its mechanism may still involve IL-4 (*26*).

In vivo T cell responses, as well as individual T cell clones, generally exhibit either a T_H1- or T_H2-like phenotype (*1–3*). This could indicate either that the natural physiologic setting during T cell activation in vivo rarely involves concurrent IL-4 and IL-12 production or that T cells are inherently unable to simultaneously produce large amounts of IL-4 and IFN-γ. T cells activated in the presence of both IL-4 and IL-12 did not acquire the capacity to secrete high concentrations of both IL-4 and IFN-γ (Fig. 3B). Rather, the IL-4 effects were dominant over those of IL-12, which inhibited the induction by IL-12 of IFN-γ (Fig. 3A) and T_H1 development (Fig. 3B). Thus, the phenomena of distinct cytokine profiles for $CD4^+$ T cells may result from an intrinsic, mutually exclusive regulation of T_H1 and T_H2 cytokine genes.

On the basis of these and other data (*12, 13*), we propose the following framework for naïve T cell activation and T_H phenotype differentiation. Macrophages are important mediators of *Listeria*-induced T_H1 development but are ineffective APCs for the stimulation of naïve T cell proliferation (*6, 13, 14*). Thus, other APCs, such as dendritic cells, are likely to be responsible for activating naïve T cells for proliferation and clonal expansion (*13, 14, 27*). Because dendritic cells are poor mediators of the effects of *Listeria* on T_H1 phenotype development (*13*), there appears to be a physiologic segregation of the ability to prime naïve T cells and the ability to skew T_H phenotype development (*13*). Future analysis of T_H phenotype development must, therefore, account for these distinct aspects of peripheral $CD4^+$ T cell development.

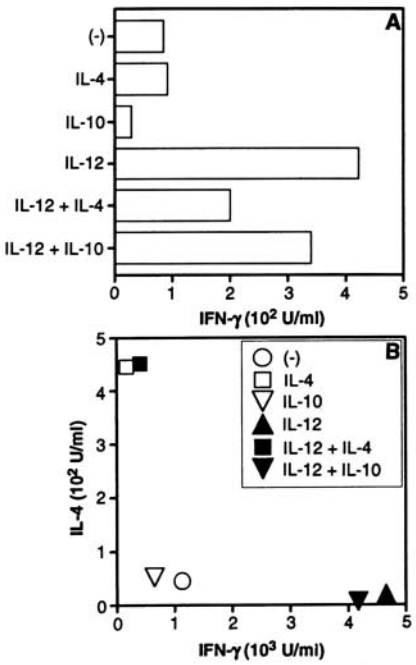

Fig. 3. Effects of IL-4 and IL-10 on IL-12–induced T_H1 development. (**A**) Effects on IFN-γ production from naïve T cells. Nylon wool–purified T cells were stimulated with OVA and irradiated BALB/c splenocytes as described in Fig. 2, C and D. Experimental conditions included additions of IL-4 (200 U/ml) (IL-4) or IL-10 (50 U/ml) (IL-10) in the absence or presence of IL-12 (10 U/ml) (IL-12) as indicated; (−) is as in Fig. 2. (**B**) Effects on T_H phenotype development. Cytokine profiles of developing transgenic T cells were assessed as described in Fig. 1. This experiment was repeated three times with similar results.

REFERENCES AND NOTES

1. T. R. Mosmann, H. M. Cherwinski, M. W. Bond, M. A. Giedlin, R. L. Coffman, *J. Immunol.* **136**, 2348 (1986); T. R. Mosmann and R. L. Coffman, *Annu. Rev. Immunol.* **7**, 145 (1989); K. Bottomly, *Immunol. Today* **9**, 268 (1988).
2. A. Sher and R. L. Coffman, *Annu. Rev. Immunol.* **10**, 385 (1992); P. Scott and S. H. E. Kaufmann, *Immunol. Today* **12**, 346 (1991); J. F. Urban *et al.*, *Immunol. Rev.* **127**, 205 (1992); A. Sher *et al.*, *ibid.*, p. 183.
3. P. Scott, P. Natovitz, R. L. Coffman, E. Pearce, A. Sher, *J. Exp. Med.* **168**, 1675 (1988); F. P. Heinzel, M. D. Sadick, B. J. Holaday, R. L. Coffman, R. M. Locksley, *ibid.* **169**, 59 (1989).
4. M. C. Kullberg, E. J. Pearce, S. E. Hieny, A. Sher, J. A. Berzofsky, *J. Immunol.* **148**, 3264 (1992).
5. K. M. Murphy, A. B. Heimberger, D. Y. Loh, *Science* **250**, 1720 (1990).
6. C.-S. Hsieh, A. B. Heimberger, J. S. Gold, A. O'Garra, K. M. Murphy, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6065 (1992).
7. S. L. Swain, A. D. Weinberg, M. English, G. Huston, *J. Immunol.* **145**, 3796 (1990); G. Le Gros, S. Z. Ben-Sasson, R. A. Seder, F. D. Finkelman, W. E. Paul, *J. Exp. Med.* **172**, 921 (1990).
8. R. A. Seder, W. E. Paul, M. M. Davis, B. F. Fazekas de St. Groth, *J. Exp. Med.* **176**, 1091 (1992).
9. D. F. Fiorentino *et al.*, *J. Immunol.* **146**, 3444 (1991).
10. S. L. Swain, G. Huston, S. Tonkonogy, A. D. Weinberg, *ibid.* **147**, 2991 (1991).
11. D. M. Magee and E. J. Wing, *ibid.* **141**, 3203 (1988); M. E. A. Mielke, S. Ehlers, H. Hahn, *Infect. Immun.* **56**, 1920 (1988); G. J. Bancroft, K. C. F. Sheehan, R. D. Schreiber, E. R. Unanue, *J. Immunol.* **143**, 127 (1989).
12. C.-S. Hsieh, S. E. Macatonia, A. O'Garra, K. M. Murphy, *Int. Immunol.*, in press.
13. S. E. Macatonia, C.-S. Hsieh, K. M. Murphy, A. O'Garra, in preparation.
14. M. Croft, D. D. Duncan, S. L. Swain, *J. Exp. Med.* **176**, 1431 (1992).
15. We have not yet addressed the potential role of other factors, such as IFN-α, steroids, or prostaglandins [F. D. Finkelman *et al.*, *ibid.* **174**, 1179 (1991); R. A. Daynes, B. A. Araneo, T. A. Dowell, K. Huang, D. Dudley, *ibid.* **171**, 979 (1990); T.-K. Li and B. S. Fox, *J. Immunol.* **150**, 1680 (1993)]. Although these factors may be involved in *Listeria*-induced T_H1 development, their contribution is likely to be small (Fig. 2F). However, they may be involved in T_H phenotype regulation of other host-pathogen interactions.
16. A. D'Andrea *et al.*, *J. Exp. Med.* **176**, 1387 (1992).
17. M. Kobayashi *et al.*, *ibid.* **170**, 827 (1989).
18. S. J. Chan *et al.*, *ibid.* **173**, 869 (1991).
19. Neutralization of TNF, which has been reported to block the effects of IL-12 on NK cell lysis [B. Naume, M. Gately, T. Espevik, *J. Immunol.* **148**, 2429 (1992)] and IFN-γ production (*20*), does not block the IL-12–dependent effects of *Listeria* (*12*). T cells and NK cells are distinct lineages and have different activation requirements. Thus, TNF may not be necessary for IL-12 effects on naïve T cells that undergo antigen activation but may be required for IL-12 stimulation of NK cells.
20. C. S. Tripp, S. F. Wolf, E. R. Unanue, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
21. D. F. Fiorentino, M. W. Bond, T. R. Mosmann, *J. Exp. Med.* **170**, 2081 (1989); R. De Waal Malefyt, J. Abrams, B. Bennett, C. G. Figdor, J. E. de Vries, *ibid.* **174**, 1209 (1991); D. F. Fiorentino, A. Zlotnik, T. R. Mosmann, M. Howard, A. O'Garra, *J. Immunol.* **147**, 3815 (1991).
22. A. Sher, D. F. Fiorentino, P. Caspar, E. Pearce, T. R. Mosmann, *J. Immunol.* **147**, 2713 (1991); J. S. Silva *et al.*, *J. Exp. Med.* **175**, 169 (1992); K. W. Moore, *Annu. Rev. Immunol.*, in press.
23. S. E. Macatonia, T. M. Doherty, S. C. Knight, A. O'Garra, in preparation.
24. C.-S. Hsieh, S. E. Macatonia, S. F. Wolf, A. O'Garra, K. M. Murphy, unpublished observations.
25. T. F. Gajewski and F. W. Fitch, *J. Immunol.* **140**, 4245 (1988); E. Maggi *et al.*, *ibid.* **148**, 2142 (1992).
26. Addition of IL-12 or anti–IL-12 with anti–IL-4 markedly enhances or inhibits, respectively, IFN-γ production during the secondary stimulation (*24*). IL-4 production, however, is unaffected by these conditions. These data suggest that the amount of IL-4 during the primary stimulation is critical for subsequent IL-4 expression by T cells and that IL-12 independently regulates the amount of IFN-γ produced. Previously, we have found that the T_H1 and T_H2 phenotypes produced by anti–IL-4 and IL-4 treatment are stable in vitro (*24*). However, we have not yet addressed the in vitro stability of T cell lines derived exclusively in the presence of IL-12. Because the events that regulate the T_H phenotype occur quite early (by day 3 during *Leishmania major* infection [P. Scott, *J. Immunol.* **147**, 3149 (1991)], our principal interest was in the short-term effects of *Listeria* and IL-12.
27. R. M. Steinman, *Annu. Rev. Immunol.* **9**, 271 (1991).
28. J. C. Wherry, R. D. Schreiber, E. R. Unanue, *Infect. Immun.* **59**, 1709 (1991).

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