Current Trends in Immunology
Vol. 13, 2012

Mini-Review

Immune memory resilience in the face of immuno-modulatory pathogens: Implications for vaccine development

Jean-Pierre Y. Scheerlinck*, Musammat F. Nahar and Alison L. Every

Centre for Animal Biotechnology, Faculty of Veterinary Science, The University of Melbourne, Parkville, 3010 Victoria, Australia

ABSTRACT

In most cases vaccination relies on the induction of immune memory responses, which are recalled during the early stages of infection. These recall responses are typically faster and stronger and have a higher affinity for the antigen, compared to primary immune responses. For many pathogens these recall immune responses represent a real challenge to their survival. As such they exert significant evolutionary pressures on pathogens resulting in survival advantages for those pathogens able to escape recall immune responses. One way of achieving this is to redirect the immune memory response away from protective immunity towards a type of immune response that is less detrimental to the pathogen. Thus some pathogens have developed immuno-modulatory properties in an attempt to circumvent immune destruction. In turn, one can expect that the immune system will also develop ways to resist immuno-modulation by pathogens. We recently introduced the concept of immune memory resilience, which is defined as the ability of an immune memory response to withstand manipulation by pathogens. In this mini-review we will examine the literature in relation to how pathogens manipulate the immune response with an emphasis on immune memory responses. We will also discuss the implication of the concept of immune memory resilience for the development of protective vaccines against immuno-modulatory pathogens.

*Corresponding author
j.scheerlinck@unimelb.edu.au

KEYWORDS: immune memory, resilience, immuno-modulation, vaccine

1. INTRODUCTION

When a vaccine is delivered to the immune system for the first time, both the antigen and adjuvant are generally quickly transported to the local lymph node [1]. Within the draining lymph node an immune response is induced and the type of immune response generated depends on the adjuvant (including the concentration of danger signal activating antigen presenting cells [2]), the route of delivery [3], the amount and intrinsic properties of the antigen delivered [4], and the genetic make-up of the vaccinated host [5].

Antigenic peptides presented in association with MHC II on the surface of dendritic cells (DCs), will activate naïve, uncommitted CD4 T cells to differentiate into distinct effector T helper (Th) cell types including: Th1, Th2, Th17 or regulatory T (Treg) cells [6]. In this process the cytokine microenvironment and expression of transcription factors that regulate cytokine gene expression plays a critical role [7, 8].

As part of that immune response generated, an immune memory is induced that will last for a prolonged period of time. For most vaccines this immune memory is critical for the protection of the vaccinated individual, as pathogens subsequently infecting their host will be faced with a rapid, elevated and highly specific immune recall response [9]. Thus in the process of vaccine development great care is taken to make sure that

an appropriate type of immune response is induced following vaccination. Indeed, it is well documented that for some diseases such as Leishmaniasis, the type of immune response induced during vaccination is critical to protection of the host and the induction of an inappropriate type of immune response can result in exacerbation of disease [10].

The induction of T cell memory can be divided into three phases. The first phase consists of the generation of memory T cells with high functional capacity, in response to specific antigen and other co-stimulatory signals. In the second stage, generated memory T cells are maintained effectively by steady-state homeostatic turnover. Finally, re-stimulation of memory T cells in an appropriate environment generates an efficient secondary response [11]. Memory T cells are thought to be derived from the effector clones [12] and during early immune induction naïve T cells can, under appropriate conditions, be "epigenetically imprinted" as Th1 or Th2 cells [13]. Memory T cells have been subdivided into central (T_{CM}) and effector (T_{EM}) memory T cells with each exerting different functions and expressing different cell surface markers (T_{CM} are $CCR7^{high}$ $CD62L^{high}$ $CD44^{high}$, while T_{EM} are $CCR7^{low}$ $CD62L^{low}$ $CD44^{high}$ [14]). The T_{CM} compartment can be thought of as the classical long-term memory T cells, which turn over very slowly and maintain immune memory for prolonged periods of time. These cells are thought to be the least differentiated and, therefore, also the most plastic in their recall responses. These cells play a key role in the long-lasting memory that vaccines rely on for induction of long-term immunity. T_{EM} cells are shorter lived and terminally differentiated.

In order for pathogens to resist being eliminated, many have developed immuno-modulatory properties. While some pathogens will change the antigenic targets as a way of escaping immune responses, others will affect the type of immune response that they induce during infection. To this end, they will down-regulate immune responses that are detrimental; one way of achieving this is to take advantage of homeostatic interactions between different arms of the immune system. For example, it is well known that Th1 and Th2 responses are mutually inhibitory by way of the cytokines they produce. So Th1 cells making IFN-γ inhibit Th2 responses [15], while Th2 cells making IL-4 and IL-10 will inhibit Th1 responses [16]. In turn, one can expect the immune system to have developed ways in which to counter these immuno-modulatory effects. The ability of the immune memory response to resist immuno-modulatory pathogens has been defined as "immune memory resilience" [17]. As shown in Fig. 1, the induction of a resilient immune memory response is critical to the outcome of an infection by a pathogen with immuno-modulatory properties. While in this illustration (Fig. 1) vaccination induces a Th1-biased immune memory response and the pathogen counters it with a Th2-inducing response, other combinations are also possible. Here we will review several key pathogens able to modulate immune responses and discuss the concept of immune memory resilience in the context of novel vaccines development against these diseases.

2. Immuno-modulatory pathogens

One of the hallmarks of successful pathogens is that they survive in their host for a sufficient period of time to allow for reproduction and transmission. Hence, many of the most successful pathogens have developed ways of counteracting immune responses of their host. There are several ways in which this can occur including: changing the antigens expressed on a regular basis and suppressing and/or modulating the immune response away from protective responses towards ineffective immune responses. We will only consider the latter as this type of immuno-modulation is most important to the concept of immune memory resilience. However, it should be noted that the concept of immune memory resilience could also be applied to the suppression of the immune response not only to its qualitative modulation. For each category of pathogens one can find documented examples of immuno-modulatory behaviour.

Viruses

As a result of co-evolution of viruses with their mammalian hosts, many viruses have developed effective ways of circumventing the immune system, often resulting in many individuals

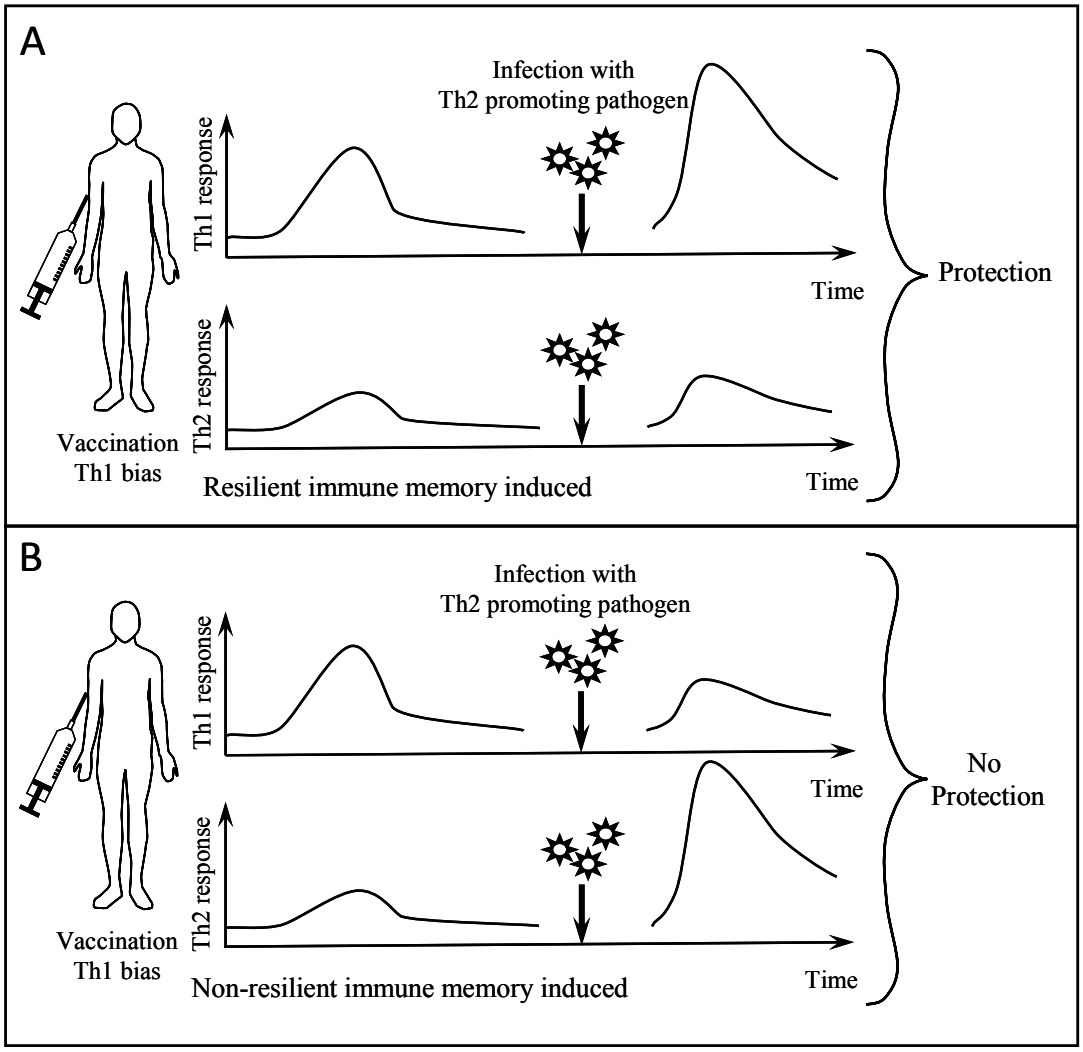

Fig. 1. Schematic representation of the implication of immune memory resilience on protection against immuno-modulatory pathogens. An individual vaccinated using a vaccine adjuvanted with a Th1 promoting adjuvant mounts a memory immune response that is biased towards Th1. The individual subsequently contracts the infection which promotes a Th2 immune response in order to escape the immune attack. If the immune memory induced during vaccination is resilient the pathogen will be unsuccessful at redirecting the immune response and the individual will be protected (A). If the vaccination did not induce a resilient immune memory response the pathogen will succeed in redirecting the recall response and will survive, so that the individual is not protected (B).

remaining infected for life. For example, viruses including leporipoxvirus, poxviruses, Epstein-Barr virus [18], human herpes virus-8 and cytomegalovirus and vaccinia, all express molecules homologous with cytokines or cytokine receptors, which have the ability to manipulate the immune system. In the case of orf virus, they produce a homologue of the immuno-modulatory protein IL-10. This molecule is a virulence factor for this virus strongly suggesting that the immuno-modulatory effect is important in viral immune evasion [19]. Conversely, evidence that the expression of cytokines by viruses can profoundly affect immune responses is suggested by studies of viral vaccine vectors overexpressing cytokines, such as IL-2, IFN-γ, IL-7, IL-4, IL-5, IL-6 and GM-CSF, chosen to improve immune responses against these vaccines [20].

Hence, virally expressed cytokines or their homologues can have profound effects on the immunogenicity of viruses. Viruses can also produce viral chemokine-binding proteins, viral chemokines and viral chemokine receptors [21]. For example, glycoprotein G (gG) is a chemokine-binding protein expressed by all α-herpesviruses that can modulate the immune response directly, by altering leukocyte trafficking to sites of infection. In bovine herpesvirus 5 [22], equine herpesviruses 1 [23], pseudorabies virus [24] and infectious laryngotracheitis virus (ILTV) [25], gG inhibits chemokine activity, which subsequently alters leukocyte migration. In contrast, gG derived from HSV-1 and -2, enhances chemokine activity [24]. Expression of chemokine receptors by memory T cells indicates that chemokines likely have an important role in recruitment, and therefore activity of memory T cells [26]. Thus herpesviruses are a prime example of how viruses evade host immunity and thus induction of immune memory. Importantly, mutant ILTV that lacks gG has proven to be an effective novel vaccine candidate [25]. Together, these findings underscore the importance of chemokine-binding proteins and other immuno-modulatory molecules in modulating immune responses, and therefore, the ability to produce a highly efficacious vaccine.

Bacteria

More complex pathogens, such as bacteria, utilise a range of strategies to subvert the immune system. For example *Chlamydia psittaci* is able to manipulate infected macrophages into producing a range of cytokines while heat killed bacteria lose this ability [27]. *Helicobacter pylori*, which can persist in the stomach for the life of the human host, can escape immune destruction through several mechanisms including: (i) avoiding immune detection by producing a low potency LPS that is poorly recognised by the innate immune system [28], (ii) reducing production of IL-12 by DCs exposed to *H. pylori* thereby redirecting immunity away from detrimental Th1 immune responses [29, 30], (iii) suppressing CD4 T-cell proliferation through the production of γ-glutamyl transpeptidase [31] and vacuolating cytotoxin (VacA) [32, 33], and (iv) induction of tolerogenic DCs and Treg that impair induction of $CD4^+$ T-cell memory, limiting the protective efficacy of vaccination [34]. Together, the strategies employed by *H. pylori* to evade host immunity explain its ability to persist for the life of the host and why successful vaccine development, to date, has proven unachievable.

Parasites

Parasites are again more complex and therefore have the opportunity to affect the immune system at many different levels. The combination of different immune evasion mechanisms makes it more difficult to identify which specific immuno-modulatory effect is critical in protecting the pathogen against the immune system.

One of the most studied parasites in the context of Th1/Th2 immune responses is Leishmania. Early on, it was recognised that Th2 responses are associated with exacerbation of disease, while Th1 responses promoted disease resolution. These early experiments were often conducted by restimulating the T cells *in vitro*, hence measuring recall responses. More recent experiments suggest that the correlation between disease outcome and type of Th cell response induced is not as clear-cut when using *ex vivo* non-restimulated T cells [35]. Hence a major effort was undertaken to switch exacerbating Th2 immune responses to protective Th1 responses. One interesting experiment used IL-12 to promote this switch in BALB/c mice, but this was only successful in combination with anti-parasitic drugs [36]. This suggests that a high parasite burden promotes Th2 responses, possibly through the induction of IL-10 by parasites infecting macrophages [37]. Leishmania infection of macrophages can also reduce macrophage responsiveness to Toll-like receptor activation, suggesting a second mechanism by which this parasite can modulate immune responses [38].

Large multi-cellular parasites with complex lifecycles regularly engage in immuno-modulatory strategies for survival. For example Schistosome parasites strongly modulate immune responses particularly during the egg stage of development [39]. Indeed, Schistosome eggs have the ability to induce very strong Th2 responses, which are largely responsible for both protection of the host against the parasite eggs as well as the pathogenic

effect of the parasite through granuloma formation [39]. The immuno-modulatory properties of Schistosome eggs has been linked to *Schistosoma mansoni* egg antigens (SmEA) or excretory/ secretory egg products, such as the omega-1 and IPSE/α-1 antigens [40]. Interestingly, vaccination of mice with whole eggs or SmEA in the presence of IL-12 leads to a Th1 response and reduction of fibrosis [41], together with a reduced Th2 response. In contrast, vaccination with eggs alone only had a moderate effect. This experiment provides direct support that the immune memory response induced through vaccination with eggs/ IL-12 induces an immune memory response that is, at least to some degree, resilient to immune manipulation by Schistosome eggs during infection.

3. Possible mechanisms of immune memory resilience

At the basis of the Th memory phenotype polarisation is the concept of genetic imprinting, resulting in cells that are committed to be polarised towards either Th1 or Th2. Thus under polarizing conditions, resilient properties of Th1 and Th2 cells can be interpreted as a result of the establishment of a stable transcriptional program [42].

The transcription factor, T-bet, functions as a master regulator of Th1 cell differentiation [43, 44]. IL-12, produced by DCs [45] can up-regulate the expression of T-bet, which, in turn, induces IFN-γ production thereby promoting Th1 differentiation. The fact that even in Th2 cells T-bet overexpression can inhibit IL-4 and stimulate IFN-γ, suggests that the imprinted program is reversible under appropriate conditions. Conversely, the absence of T-bet in T-bet$^{-/-}$ cells results in a failure to differentiate into Th1 cells and as a result, T-bet$^{-/-}$ mice spontaneously develop asthma-like diseases [46]. The transcription factor, GATA-3, is the principal regulator of Th2 cell differentiation. Naïve CD4 T cells can produce limited amounts of IL-4, which up-regulates GATA-3 expression [47]. GATA-3 overexpression in Th1 cells induces IL-4 production and in the absence of GATA-3, Th2 differentiation is completely abolished both *in vitro* and *in vivo* [48]. There is also evidence that in fully differentiated Th2 cells, deletion of GATA-3 completely stops the subsequent production of IL-5 and IL-13 [48]. Hence suggesting that, given appropriate stimuli Th2 cells might be re-directed towards a Th1 phenotype. Thus it cannot be excluded that even fully differentiated Th-phenotypes might change under extreme conditions and it is now recognised that the Th-phenotypes are more plastic than initially thought [49]. Resisting this plasticity during immune memory re-stimulation is the basis of immune memory resilience.

Recent studies are now also highlighting the importance of the Th17 phenotype for induction of immunity, particularly to extracellular pathogens. Importantly, epigenetic mapping has revealed that the Th17 phenotype, induced under the control of RORγt transcription factor [50], is somewhat less stable than the Th1 and Th2 phenotypes [49].

However, in thinking about ways in which Th responses can switch phenotypes it is also useful to consider the Th response at a cell population level. Hence if a pathogen is able to inhibit proliferation of one type of Th cell and promote the proliferation of the opposite type, over time one could expect that the later type would come to dominate even if no conversion is induced.

4. Measuring immune memory resilience for optimising vaccines against immuno-modulatory pathogens

Most of the recombinant and killed vaccines use adjuvants to increase and direct the immune response to the antigen. The adjuvants therefore play a critical role in defining the type of immune response that will be induced. In addition, there is now mounting evidence that adjuvants also have the ability to shape the immune repertoire [51]. Thus adjuvants are critical to the success of many vaccines. However, so far in the process of developing vaccines, the selection of the adjuvant was largely determined by their ability to induce elevated immune responses and to direct the immune response towards a type of immune response that correlates with protective immunity. In considering immunisation against immuno-modulatory pathogens, and taking into account the plasticity in redirecting immune recall responses,

it is now essential to also consider whether the induced immune response will be able to resist the immuno-modulatory properties of the pathogen (i.e. how resilient is the immune memory response induced). However, the parameters that affect immune memory resilience are poorly understood and may be very different from those that induce elevated responses. Hence, there is a need to determine key parameters that affect immune memory resilience and to incorporate these findings in the development of vaccination strategies against immuno-modulatory pathogens.

While the concept of immune memory resilience is straightforward, designing a suitable way to measure the degree of resilience of the immune memory induced following vaccination is more complex. One major difficulty is that there are many ways in which pathogens can interfere with the immune recall responses and that it is likely that the susceptibility of the immune memory to different immuno-modulatory signals may be very different. In addition, as highlighted in this review, complex pathogens often interact with the immune system at many different levels and employ a combination of escape mechanisms. Hence, the picture rapidly becomes complicated and needs to be simplified in order to unravel basic principles governing the induction of resilient immune memory responses. One way of achieving this is to employ adjuvants (in a broad sense and including cytokines), as simple immuno-modulators and define the principles based on experiments in which a succession of adjuvants is used to direct the immune response in different ways. For example one could use a Th2-inducing adjuvant such as alum to generate an immune memory response against a model antigen, and challenge this immune response *in vivo* using a stimulus known to bias the immune response towards a Th1 response. The degree to which the recall response can resist being redirected towards the Th1 response provides information about the resilience of the immune memory response generated during the priming with the Th2 inducing adjuvant. The less the immune recall response can be changed the more effective the vaccination strategy was at inducing a resilient immune memory.

CONCLUSION

Although much of the transcriptional program of the Th cell phenotypes is regulated by epigenetic modifications to genes associated with these phenotypes, there is likely more flexibility in the cytokines expressed by individual cells than the paradigm suggests. Indeed, a systematic analysis of how resilient these phenotypes are to manipulation on single-cell versus a population level may lead to design of improved vaccines that ensure Th phenotypes are less easily manipulated by immuno-modulatory pathogens. In our quest to develop more effective vaccines, the search for vaccination strategies for inducing robust immune memory responses must include ways of inculcating these responses with the resilience that will allow them to resist manipulation by pathogens.

ACKNOWLEDGMENT

This work was supported by the Australian Research Council.

REFERENCES

1. Windon, R. G., Chaplin, P. J., McWaters, P., Tavarnesi, M., Tzatzaris, M., Kimpton, W., Cahil, R., Beezum, L., Coulter, A., Drane, D., Sjolander, A., Pearse, M., Scheerlinck, J.-P. Y. and Tennent, J. 2001, Vaccine, 20, 490.
2. Peters, M., Dudziak, K., Stiehm, M. and Bufe, A. 2010, Immunol. Cell Biol., 88, 537.
3. Liew, F. Y., Hale, C. and Howard, J. G. 1985, J. Immunol., 135, 2095.
4. DeKruyff, R. H., Fang, Y. and Umetsu, D. T. 1992, J. Immunol., 149, 3468.
5. Beebe, A. M., Cua, D. J. and Coffman, R. L. 1999, Microbes and infection/Institut Pasteur, 1, 89.
6. Wang, X. and Mosmann, T. 2001, J. Exp. Med., 194, 1069.
7. Bluestone, J. A., Mackay, C. R., O'Shea, J. J. and Stockinger, B. 2009, Nature reviews. Immunology, 9, 811.
8. Murphy, K. M. and Stockinger, B. 2010, Nature Immunology, 11, 674.
9. Sallusto, F., Lanzavecchia, A., Araki, K. and Ahmed, R. 2010, Immunity, 33, 451.

10. Titus, R. G., Lima, G. C., Engers, H. D. and Louis, J. A. 1984, J. Immunol., 133, 1594.
11. Lees, J. R. and Farber, D. L. 2010, Immunology, 130, 463.
12. Harrington, L. E., Janowski, K. M., Oliver, J. R., Zajac, A. J. and Weaver, C. T. 2008, Nature, 452, 356.
13. Ansel, K. M., Lee, D. U. and Rao, A. 2003, Nat. Immunol., 4, 616.
14. Bjorkdahl, O., Barber, K. A., Brett, S. J., Daly, M. G., Plumpton, C., Elshourbagy, N. A., Tite, J. P. and Thomsen, L. L. 2003, Immunology, 110, 170.
15. Gajewski, T. F. and Fitch, F. W. 1988, J. Immunol., 140, 4245.
16. Fiorentino, D. F., Zlotnik, A., Vieira, P., Mosmann, T. R., Howard, M., Moore, K. W. and O'Garra, A. 1991, J. Immunol., 146, 3444.
17. Scheerlinck, J.-P. Y. and Yen, H. H. 2010, Expert Rev. Vaccines, 9, 351.
18. Moore, K. W., Vieira, P., Fiorentino, D. F., Trounstine, M. L., Khan, T. A. and Mosmann, T. R. 1990, Science, 248, 1230.
19. Fleming, S. B., Anderson, I. E., Thomson, J., Deane, D. L., McInnes, C. J., McCaughan, C. A., Mercer, A. A. and Haig, D. M. 2007, J. Gen. Virol., 88, 1922.
20. Bukreyev, A. and Belyakov, I. M. 2002, Expert Review of Vaccines, 1, 233.
21. Alcami, A. and Lira, S. A. 2010, Curr. Opin. Immunol., 22, 482.
22. Bryant, N. A., Davis-Poynter, N., Vanderplasschen, A. and Alcami, A. 2003, The EMBO Journal, 22, 833.
23. Van de Walle, G. R., May, M. L., Sukhumavasi, W., von Einem, J. and Osterrieder, N. 2007, J. Immunol., 179, 4161.
24. Viejo-Borbolla, A., Munoz, A., Tabares, E. and Alcami, A. 2010, The Journal of General Virology, 91, 23.
25. Devlin, J. M., Viejo-Borbolla, A., Browning, G. F., Noormohammadi, A. H., Gilkerson, J. R., Alcami, A. and Hartley, C. A. 2010, Vaccine, 28, 1325.
26. Sallusto, F., Mackay, C. R. and Lanzavecchia, A. 2000, Annu. Rev. Immunol., 18, 593.
27. Entrican, G., Wilkie, R., McWaters, P., Scheerlinck, J., Wood, P. R. and Brown, J. 1999, Clin. Exp. Immunol., 117, 309.
28. Muotiala, A., Helander, I. M., Pyhala, L., Kosunen, T. U. and Moran, A. P. 1992, Infect. Immun., 60, 1714.
29. Kao, J. Y., Rathinavelu, S., Eaton, K. A., Bai, L., Zavros, Y., Takami, M., Pierzchala, A. and Merchant, J. L. 2006, Am. J. Physiol. Gastrointest. Liver Physiol., 291, G73.
30. Mitchell, P., Germain, C., Fiori, P. L., Khamri, W., Foster, G. R., Ghosh, S., Lechler, R. I., Bamford, K. B. and Lombardi, G. 2007, Infect. Immun., 75, 810.
31. Schmees, C., Prinz, C., Treptau, T., Rad, R., Hengst, L., Voland, P., Bauer, S., Brenner, L., Schmid, R. M. and Gerhard, M. 2007, Gastroenterology, 132, 1820.
32. Boncristiano, M., Paccani, S. R., Barone, S., Ulivieri, C., Patrussi, L., Ilver, D., Amedei, A., D'Elios, M. M., Telford, J. L. and Baldari, C. T. 2003, J. Exp.Med., 198, 1887.
33. Gebert, B., Fischer, W., Weiss, E., Hoffmann, R. and Haas, R. 2003, Science, 301, 1099.
34. Hitzler, I., Oertli, M., Becher, B., Agger, E. M. and Muller, A. 2011, Gastroenterology, 141, 186.
35. Choi, B. S. and Kropf, P. 2009, Parasite Immunol., 31, 199.
36. Scott, P. 1998, Immunol. Res., 17, 229.
37. Nandan, D., Camargo de Oliveira, C., Moeenrezakhanlou, A., Lopez, M., Silverman, J. M., Subek, J. and Reiner, N. E. 2012, J. Immunol., 188, 367.
38. Das, S., Pandey, K., Kumar, A., Sardar, A. H., Purkait, B., Kumar, M., Kumar, S., Ravidas, V. N., Roy, S., Singh, D. and Das, P. 2012, Immunol. Cell Biol., 90, 640.
39. Wilson, M. S., Mentink-Kane, M. M., Pesce, J. T., Ramalingam, T. R., Thompson, R. and Wynn, T. A. 2007, Immunol. Cell Biol., 85, 148.
40. Schramm, G. and Haas, H. 2010, Microbes and infection/Institut Pasteur, 12, 881.
41. Wynn, T. A., Cheever, A. W., Jankovic, D., Poindexter, R. W., Caspar, P., Lewis, F. A. and Sher, A. 1995, Nature, 376, 594.
42. Grogan, J. L., Mohrs, M., Harmon, B., Lacy, D. A., Sedat, J. W. and Locksley, R. M. 2001, Immunity, 14, 205.
43. Zygmunt, B. and Veldhoen, M. 2011, Adv. Immunol., 109, 159.

44. Szabo, S. J., Kim, S. T., Costa, G. L., Zhang, X., Fathman, C. G. and Glimcher, L. H. 2000, Cell, 100, 655.
45. Trinchieri, G. and Sher, A. 2007, Nature reviews. Immunology, 7, 179.
46. Finotto, S., Neurath, M. F., Glickman, J. N., Qin, S., Lehr, H. A., Green, F. H., Ackerman, K., Haley, K., Galle, P. R., Szabo, S. J., Drazen, J. M., De Sanctis, G. T. and Glimcher, L. H. 2002, Science, 295, 336.
47. Zhu, J. and Paul, W. E. 2008, Blood, 112, 1557.
48. Zhu, J., Min, B., Hu-Li, J., Watson, C. J., Grinberg, A., Wang, Q., Killeen, N., Urban, J. F. Jr., Guo, L. and Paul, W. E. 2004, Nature Immunology, 5, 1157.
49. Kanno, Y., Vahedi, G., Hirahara, K., Singleton, K. and O'Shea, J. J. 2012, Annu. Rev. Immunol., 30, 707.
50. Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., Cua, D. J. and Littman, D. R. 2006, Cell, 126, 1121.
51. Baumgartner, C. K. and Malherbe, L. P. 2010, Immunology, 130, 16.