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Cutting Edge Commentary: Immune Responses in the Absence of Costimulation: Viruses Know the Trick[1]

Martin F. Bachmann,[2]* Rolf M. Zinkernagel,† and Annette Oxenius†

Costimulatory molecules are crucial for the induction of immune responses after immunization with purified proteins or peptides. However, some viruses and other pathogens are able to induce protective immunity in the absence of such molecules. This review argues that patterns recognized by both the specific and the innate immune system, together with a high and sustained Ag-load, are responsible for these surprisingly efficient immune responses triggered by pathogens. ***The Journal of Immunology*****, 1998, 161: 5791–5794.**

A large part of the fundamentals of current immunology have been established using purified model Ags, such as lysozyme and OVA. However, to induce an immune response, administration of such purified proteins alone is usually not sufficient, and the Ag has to be given together with helper substances, called adjuvants. The mechanism that governs how these helper substances work is poorly understood at the present time. Unlike isolated model Ags such as OVA, viruses induce prompt and efficient B and T cell responses in the absence of adjuvants. Moreover, although viruses often consist of few proteins only, they are able to trigger much stronger immune responses than their isolated components. This review discusses two important factors that are responsible for the efficient immune responses usually associated with pathogens: 1) invariant patterns present on pathogens link the innate to the specific immune system, and 2) Ag kinetics, load, and distribution differ between pathogens and model Ags.

Since different mechanisms govern activation of B vs T cells, the two types of responses will be discussed separately.

Pathogen-Specific B Cell Responses

Most viruses and bacteria induce potent B cell responses, and virus-specific IgM Abs are often induced in the absence of Th cells (1). One crucial factor for this superb immunogenicity of viruses is the organization of surface antigenic epitopes. Many viruses exhibit a quasi-crystalline surface that efficiently cross-links specific Igs on B cells, which greatly facilitates B cell activation (1). Therefore, viral proteins expressed in a repetitive and ordered fashion on the viral surface are often much more immunogenic than in soluble form (2–4). Thus, B cells recognize organized and rigid surfaces as a stimulatory pattern. Interestingly, this type of pattern recognition is mediated by the B cell receptor and is therefore directly linked to the specific immune system (Fig. 1).

Coengagement of the CD19/CD21/TAPA-1 complex with the B cell receptor leads to a reduced activation threshold of B cells. CD21 is the receptor for degradation products of the C3 component of the complement cascade, and C3d has been shown to function as a molecular adjuvant if coupled to Ags (5). Since the C3 degradation products preferentially decorate the surface of pathogens (6), the CD19/CD21/TAPA-1 complex relays the complement cascade to specific recognition of Ags by B cells (7). Thus, it serves as a bridge between innate immunity and specific recognition. Two interesting points should be emphasized: 1) Both the recognition of Ag organization and the CD21-mediated augmentation of B cell responses function via the B cell receptor; i.e., the pattern recognition machinery is directly linked to the Ag-recognition machinery (Fig. 1*A*). Interestingly, this link may not always be positive because B cell activation by RP-105, a toll-like protein expressed on B cells that is likely involved in pattern recognition, is inhibited by B cell receptor signaling (8). 2) Both systems function independently, i.e., Ag organization augments B cell responses in the absence of a functional CD19/CD21/TAPA-1 complex (9) and vice versa, CD21 can enhance B cell responses to soluble Ags (5).

Pathogen-Specific T Cell Responses

It has been known for quite some time that viruses and other pathogens induce much stronger T cell responses than model Ags (10). However, the recent generation of a variety of gene-deficient mice has reinforced the interest in antiviral immunity. Much to the surprise of immunologists, mice deficient for molecules previously thought to be essential for the immune system mounted nearly normal responses after infection with some viruses. Thus, lymphocytic choriomeningitis

*Basel Institute for Immunology, Basel, Switzerland; and †Institute for Experimental Immunology, Zürich, Switzerland

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[2] Address correspondence and reprint requests to Dr. Martin F. Bachmann, Basel Institute for Immunology, Grenzacherstrasse 487, 4005 Basel, Switzerland. E-mail address: bachmann@bii.ch

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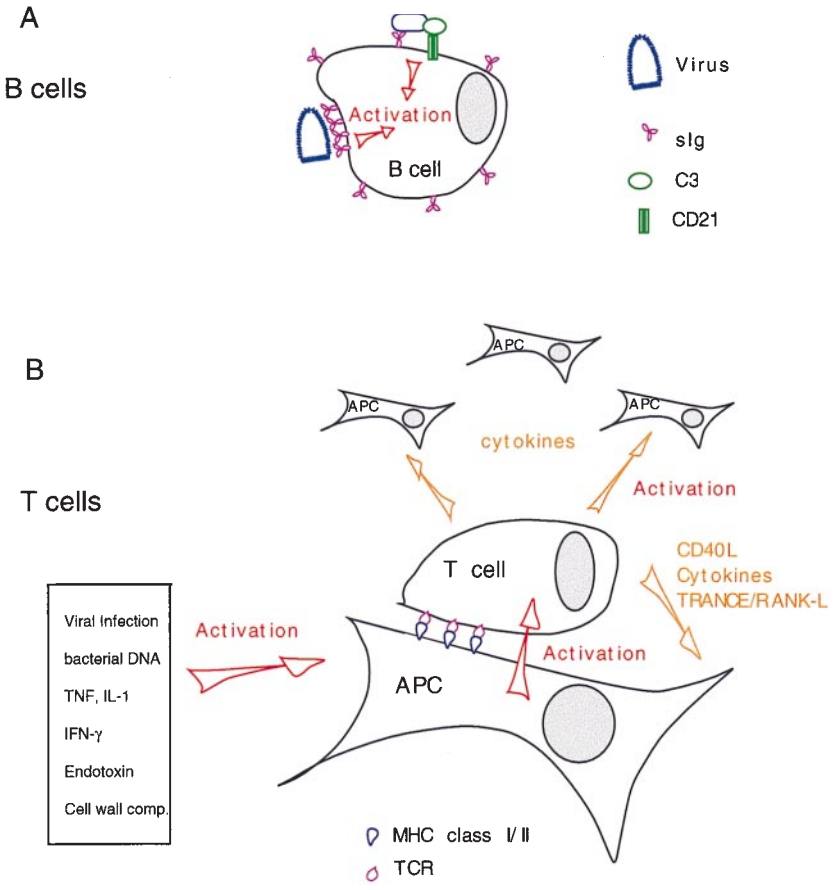

FIGURE 1. Pattern recognition by B cells (*A*) and T cells (*B*).

virus (LCMV)[3] and vaccinia virus (VV) were able to induce strong Th and CTL responses in the absence of, for example, CD28, LFA-1, or CD40 (11–13). These results were surprising, since previous experiments using model Ags had indicated that T cell activation and proliferation were critically dependent on these molecules. Since CD28 and LFA-1 are expressed on T cells, while CD40 is expressed on APCs, they represent two different classes of accessory molecules that will be considered separately.

Although LFA-1 has been shown to be important in allospecific T cell responses and has also been suggested to serve as a costimulatory molecule for T cells (14), LFA-1-deficient mice mounted almost normal virus-specific CTL responses (12). Subsequent analysis of LFA-1-deficient mice expressing a transgenic TCR specific for LCMV showed that LFA-1 assists T cell activation by promoting T cell-APC adhesion and does not measurably interfere with T cell activation otherwise (15). Moreover, the presence of LFA-1 was only critical when peptide densities on APCs were limiting. However, peptide densities on APCs reached after viral infections are usually high, which explains why the presence of LFA-1 is not limiting for effective antiviral immune responses (15).

Some viruses are able to induce CD28-independent T cell responses (11, 16, 17). Similarly, *Leishmania* induces an efficient and protective immune response in the absence of CD28 (18, 19). How can the CD28 independence of these responses be explained? CD28 amplifies TCR-mediated signals and reduces thresholds required for T cell activation (15, 20, 21). However, although CD28 is critical for activation of T cells after stimulation with low affinity ligands, naive CD28-deficient T cells can efficiently be triggered both in vitro and in vivo by high affinity ligands (15, 16, 22). Since many pathogen-derived epitopes are recognized with high affinity by T cells, they are able to initially activate T cells in the absence of CD28. However, high affinity peptides are unable to trigger sustained responses of CD28-deficient TCR-transgenic T cells in vitro and in vivo (15, 16, 22). On the contrary, T cells become unresponsive or die after antigenic stimulation in the absence of CD28 (23). Thus, not so much the initial activation of T cells (signals 2c), but rather the induction of long-lived and sustained T cell responses (signal 2t) (15) by pathogens in the absence of CD28 deserves further explanation. Interestingly, only widely replicating viruses such as LCMV or VV were able to generate CTL responses in CD28-deficient mice, while abortively replicating vesicular stomatitis virus or attenuated VV failed to do so (16). Moreover, only continuous application of peptide to TCR-transgenic mice was able to induce a long-lived response (16). Thus, the sustained presence of Ag seems to be an important factor for the CD28 independence of T cell responses. However, additional factors are likely to contribute. The availability of IL-2 seems to be critical for the outcome of T cell stimulation in the absence of CD28, since addition of exogenous IL-2 often overcomes limitations due to the absence of CD28. Thus, it is possible that during infections, levels of IL-2 are reached in vivo that subsequently render the response independent of CD28; responses that fail to reach this threshold remain abortive, while responses become practically normal once the threshold is reached. Sustained presence of Ag will obviously facilitate to reach this threshold. In addition, as

[3] Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; VV, vaccinia virus; DCs, dendritic cells.

discussed below, pathogen-specific patterns recognized by the innate immune system enhance expression of various accessory molecules on APCs that may replace CD28.

CD40-CD40 ligand (CD40L) interaction has been shown to be essential for induction of Th cell responses after immunization with protein in adjuvants (24). In contrast, LCMV- (13) and *Histoplasma capsulatum* (25)-specific Th1 responses were induced in the absence of CD40. Interestingly, CD40 and CD40L-deficient mice failed to mount protective anti-*Leishmania* Th cell responses (26–28), and CD40 also proved to be essential for protective Th1 responses against *Pneumocystis carinii* (29) and *Cryptosporidium parvum* (30). Thus, not all pathogens were able to trigger CD40-independent Th1 responses. Moreover, while vesicular stomatitis virus failed to induce T cell responses in the absence of CD28 (16), it readily did so in the absence of CD40 (13, 31). In contrast, *Leishmania* triggered protective T cell responses in the absence of CD28 (18, 19) but failed to do so in the absence of CD40 (26–28). Thus, different factors seem to determine whether CD28 and CD40 are required for the induction of pathogen-specific T cell responses.

Although various components are probably involved in the induction of T cell responses in the absence of CD40, activation of the innate immune system is likely to be critical. CD40 engagement has been shown to efficiently activate macrophages, dendritic cells (DCs), and other APCs for the up-regulation of costimulatory molecules, the enhanced expression of class II molecules, and the production of IL-12, a critical cytokine for the development of Th1 responses (32, 33). In addition, CD40 has been shown to be involved in the generation of CTL responses to model Ags (34–36). Interestingly, similar to CD40, many structures and invariant patterns (also called pathogen-associated molecular patterns (37)) present on pathogens, such as fungal and bacterial cell wall components including LPS, dsRNA on viruses, and unmethylated CpG rich motifs in bacterial, fungal, and possibly viral DNA are able to activate the production of type I IFNs, expression of costimulatory molecules, and up-regulate expression of class II on DCs and macrophages (32, 33) (Fig. 1*B*). They also trigger the migration of DCs from peripheral tissues to secondary lymphoid organs. Moreover, viral infection has been shown to directly increase expression of costimulatory molecules on splenic APCs, and to enhance immunogenicity of DCs in vitro, providing at least one explanation for why viruses can induce T cell responses in the absence of CD40 (34, 38). This increased immunogenicity may be due to the presence of viral DNA/RNA and cell debris or apoptotic cells induced by the infection (39, 40). As an additional mechanism, pathogens may be able to stimulate expression of accessory molecules on APCs that are able to replace CD40. TRANCE receptor (RANK) is a likely candidate because it exhibits high homology to CD40 and is able to augment the costimulatory capacity of DCs and also promote their survival (41, 42). Thus, induction of TRANCE receptor/RANK expression on APCs after infection may be responsible for CD40-independent T cell activation.

In addition, widespread activation of APCs in lymphoid organs occurs during the course of an immune response due to a positive feedback mechanism (C. Ruedl and M. F. Bachmann, unpublished data). As already discussed, patterns recognized on pathogens lead to the activation of APCs. These activated APCs are able to efficiently stimulate T cells that, in turn, secrete inflammatory cytokines/chemokines, which may lead to the activation of neighboring APCs (Fig. 1*B*). Similarly, as described for CD28, pathogens may therefore be able to create an immunological condition that leads to generalized, poorly controlled activation of APCs. This up-regulation of accessory molecules and secretion of cytokines by both T cells and APCs may render the response little dependent on the presence of one particular cytokine or costimulatory molecule. A change in chemokine and chemokine receptor expression induced by inflammatory cytokines (43) may further enhance the immunostimulatory capacity of the lymphoid environment.

Although little is known about innate immune responses to parasites such as *Leishmania*, it is likely that fewer invariable structures, such as unmethylated CpGs, or cell wall components, such as LPS, are present on these eukaryotic cells than on viruses, bacteria, or fungi. Moreover, surface structures on parasites are less repetitive than on viruses and bacteria. These considerations offer an explanation for the requirement of CD40 for *Leishmania*-specific Th cell responses. In addition, since T cells are involved in the activation of APCs, it is likely that local inflammatory responses outside secondary lymphoid organs, where few T cells are present during the course of the immune response, are more dependent on the presence of particular molecules, such as CD40, than responses occurring within T cell-rich lymphoid organs. This may explain why CD40 is essential for elimination of *P. carinii* (29) and *C. parvum* (30) from the lung but not for the generation of Th1 responses in the spleen after infection with LCMV (13).

Interestingly, T cells themselves do not respond to these nonspecific stimuli. Thus, in contrast to pattern recognition by B cells that function via the B cell receptor, pattern recognition by T cells occurs via the APC. (Fig. 1*B*). The explanation for this difference lies in the way the two cell types recognize their respective Ags. While B cells interact directly with native Ags, conventional $\alpha\beta$ T cells interact with peptides presented by MHC Ags. Thus, T cell-mediated recognition is restricted and is therefore unable to directly react to pathogen-associated patterns. It remains possible that unconventional T cells and possibly $\gamma\delta$ T cells may directly recognize patterns. Surprisingly, and in contrast to T cells, B cells are susceptible to stimulation with LPS or bacterial DNA. Since the probability to activate a B cell expressing a receptor with specificity for the infecting agent using nonspecific mechanisms is very low, the biological advantage of B cells recognizing patterns that are not linked to the B cell receptor is not immediately evident. However, LPS-mediated B cell activation does not necessarily occur unspecifically but happens preferentially for B cells recognizing Ags coupled to LPS, such as bacterial cell walls (44). Thus, similar to CD19/CD21/TAPA-1, LPS may lower the activation threshold of B cells and focus their response on Ags associated with LPS. Due to MHC restriction, such a mechanism is again not feasible for T cells.

These considerations may have implications for the design of vaccines. For optimal immunogenicity, vaccines should carry the signature of pathogens. Thus, they should mimic viral surfaces and allow the fixation of complement components, be delivered in a localized and sustained fashion, and possibly carry patterns recognized by the innate immune system, such as CpG-rich unmethylated DNA.

In conclusion, many pathogens are able to trigger immune responses that are largely independent of costimulation, since 1) the local Ag load is high and sustained after infection, and 2) patterns recognized on pathogens create a immunostimulatory environment that overrides the selective dependence of the response on particular accessory molecules and cytokines.

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References

1. Bachmann, M. F., and R. M. Zinkernagel. 1997. Neutralizing anti-viral B cell responses. *Annu. Rev. Immunol. 15:235.*
2. Bachmann, M. F., R. U. Hoffmann, T. M. Kündig, K. Bürki, H. Hengartner, and R. M. Zinkernagel. 1993. The influence of antigen organization on B cell responsiveness. *Science 262:1448.*
3. Justewicz, D. M., P. C. Doherty, and R. G. Webster. 1995. The B-cell response in lymphoid tissue of mice immunized with various antigenic forms of the influenza virus hemagglutinin. *J. Virol. 69:5414.*
4. Szomolanyi-Tsuda, E., Q. P. Le, R. L. Garcea, and R. M. Welsh. 1998. T cell independent immunoglobulin G responses in vivo are elicited by live virus infection but not by immunization with viral proteins or virus-like particles. *J. Virol. 72:6665.*
5. Dempsey, P. W., M. E. Allison, S. Akkaraju, C. C. Goodnow, and D. T. Fearon. 1996. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science 271:348.*
6. Müller-Eberhard, H. J. 1988. Molecular organization and function of the complement system. *Annu. Rev. Biochem. 57:321.*
7. Carroll, M. C., and A. P. Prodeus. 1998. Linkages of the innate and adaptive immunity. *Curr. Opin. Immunol. 10:36.*
8. Chan, V. W. F., I. Mecklenbräuker, I. Su, G. Texido, M. Leitges, R. Carsetti, C. A. Lowell, K. Rajewsky, K. Miyake, and A. Tarakhovsky. 1998. The molecular mechanism of B cell activation by toll-like receptor protein RP-105. *J. Exp. Med. 188:93.*
9. Fehr, T., R. C. Rickert, B. Odermatt, J. Roes, K. Rajewsky, H. Hengartner, and R. M. Zinkernagel. 1998. Antiviral protection and germinal center formation, but impaired B cell memory in the absence of CD19. *J. Exp. Med. 188:145.*
10. Janeway, C. 1989. *Approaching the Asymptote? Evolution and Revolution in Immunology.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; pp. 1–13.
11. Shahinian, A., K. Pfeffer, K. P. Lee, T. M. Kündig, P. S. Ohashi, C. B. Thompson, and T. W. Mak. 1993. Differential T cell costimulatory requirements in CD28-deficient mice. *Science 261:609.*
12. Schmits, R., T. M. Kündig, D. Baker, G. Shumaker, J. J. L. Smard, G. Duncan, A. Wakeham, A. Shahinian, A. van der Heiden, M. F. Bachmann, P. Ohashi, T. W. Mak, and D. D. Hickstein. 1996. LFA-1-deficient mice show normal CTL responses to virus but fail to reject immunogenic tumor. *J. Exp. Med. 183:1415.*
13. Oxenius, A., K. A. Campbell, C. R. Maliszewski, T. Kishimoto, H. Kikutani, H. Hengartner, R. M. Zinkernagel, and M. F. Bachmann. 1996. CD40-CD40 ligand interactions are critical in T-B cooperation but not for other anti-viral $CD4^+$ T cell functions. *J. Exp. Med. 183:2209.*
14. Springer, T. A. 1990. Adhesion receptors of the immune system. *Nature 346:425.*
15. Bachmann, M. F., K. McKall-Faienza, R. Schmits, D. Bouchard, J. Beach, D. E. Speiser, T. W. Mak, and P. S. Ohashi. 1997. Distinct roles for LFA-1 and CD28 during activation of naive T cells: adhesion versus costimulation. *Immunity 7:549.*
16. Kündig, T. M., A. Shahinian, K. Kawai, H.-W. Mittrücker, E. Sebzda, M. F. Bachmann, T. W. Mak, and P. S. Ohashi. 1996. Duration of TCR stimulation determines costimulatory requirements. *Immunity 5:41.*
17. Champagne, E., L. Scarpellino, P. Lane, and H. Acha-Orbea. 1996. CD28/CTLA4–B7 interaction is dispensable for T cell stimulation by mouse mammary tumor virus superantigen but not for B cell differentiation and virus dissemination. *Eur. J. Immunol. 26:1595.*
18. Corry, D. B., S. L. Reiner, P. S. Linsley, and R. M. Locksley. 1994. Differential effects of blockade of CD28–B7 on the development of Th1 or Th2 effector cells in experimental leishmaniasis. *J. Immunol. 153:4142.*
19. Brown, D. R., J. M. Green, N. H. Moskowitz, M. Davis, C. B. Thompson, and S. L. Reiner. 1996. Limited role of CD28-mediated signals in T helper subset differentiation. *J. Exp. Med. 184:803.*
20. Viola, A., and A. Lanzavecchia. 1996. T cell activation determined by T cell receptor number and tunable thresholds. *Science 273:104.*
21. Tuosto, L., and O. Acuto. 1998. CD28 affects the earliest signaling events generated by TCR engagement. *Eur. J. Immunol. 28:2131.*
22. Lucas, P. J., I. Negishi, K. Nakayama, L. E. Fields, and D. Y. Loh. 1995. Naive CD28-deficient T cells can initiate but not sustain an in vitro antigen-specific immune response. *J. Immunol. 154:5757.*
23. Lenschow, D. J., T. L. Walunas, and J. Bluestone. 1996. CD28/B7 system of T cell costimulation. *Annu. Rev. Immunol. 14:259.*
24. Grewal, I. S., and R. A. Flavell. 1998. CD40 and CD154 in cell-mediated immunity. *Annu. Rev. Immunol. 16:111.*
25. Zhou, P., and R. A. Seder. 1998. CD40 ligand is not essential for induction of type 1 cytokine responses or protective immunity after primary or secondary infection with histoplasma capsulatum. *J. Exp. Med. 187:1315.*
26. Campbell, K. A., P. J. Ovendale, M. K. Kennedy, W. C. Fanslow, S. G. Reed, and C. R. Maliszewski. 1996. CD40 ligand is required for protective cell-mediated immunity to *Leishmania major*. *Immunity 4:283.*
27. Kamanaka, M., P. Yu, T. Yasui, K. Yoshida, T. Kawabe, T. Horii, T. Kishimoto, and H. Kikutani. 1996. Protective role of CD40 in *Leishmania major* infection at two distinct phases of cell-mediated immunity. *Immunity 4:275.*
28. Soong, L., J. C. Xu, I. S. Grewal, P. Kima, J. Sun, B. J. Longley, Jr., N. H. Ruddle, D. McMahon-Pratt, and R. A. Flavell. 1996. Disruption of CD40-CD40 ligand interactions results in an enhanced susceptibility to *Leishmania amazonensis* infection. *Immunity 4:263.*
29. Wiley, J. A., and A. G. Harmsen. 1995. CD40 ligand is required for resolution of *Pneumocystis carinii* pneumonia in mice. *J. Immunol. 155:3525.*
30. Cosyns, M., S. Tsirkin, M. Jones, R. Flavell, H. Kikutani, and A. R. Hayward. 1998. Requirement of CD40-CD40 ligand interaction for elimination of *Cryptosporidium parvum* from mice. *Infect. Immun. 66:603.*
31. Borrow, P., A. Tishon, S. Lee, J. Xu, I. S. Grewal, M. B. Oldstone, and R. A. Flavell. 1996. CD40L-deficient mice show deficits in antiviral immunity and have an impaired memory $CD8^+$ CTL response. *J. Exp. Med. 183:2129.*
32. Cella, M., F. Sallusto, and A. Lanzavecchia. 1997. Origin, maturation and antigen-presenting function of dendritic cells. *Curr. Opin. Immunol. 9:10.*
33. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245.*
34. Ridge, J. P., F. DiRosa, and P. Matzinger. 1998. A conditional dendritic cell can be a temporal bridge between a $CD4^+$ T helper and a T killer cell. *Nature 393:474.*
35. Bennett, S. R. M., F. R. Carbone, F. Karamalis, R. A. Flavell, J. F. A. P. Miller, and W. R. Heath. 1998. Help for cytotoxic T cell responses is mediated by CD40 signalling. *Nature 393:478.*
36. Schoenenberger, S. P., R. E. M. Toes, E. I. H. vanderVoort, R. Offringa, and C. J. M. Melief. 1998. T cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature 393:480.*
37. Medzitov, R., and C. A. Janeway. 1997. Innate Immunity: impact on the adaptive immune response. *Curr. Opin. Immunol. 9:4.*
38. Wu, Y., and Y. Liu. 1994. Viral induction of co-stimulatory activity on antigen-presenting cells bypasses the need for $CD4^+$ T-cell help in $CD8^+$ T-cell responses. *Curr. Biol. 4:499.*
39. Bevan, M. J. 1987. Class discrimination in the world of immunology. *Nature 325:192.*
40. Albert, M. L., B. Sauter, and N. Bhardwaj. 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature 392:86.*
41. Wong, B. R., R. Josien, S. Y. Lee, B. Sauter, H.-L. Li, R. M. Steinman, and Y. Choi. 1997. TRANCE, a new TNF family member predominantly expressed in T cells, is a dendritic cell specific survival factor. *J. Exp. Med. 186:2075.*
42. Anderson, D. M., E. Maraskovsky, W. L. Billingsley, W. C. Dougall, M. E. Tometsko, E. R. Roux, M. C. Teepe, R. F. DuBose, D. Cosman, and L. Galibert. 1997. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature 390:175.*
43. Sallusto, F., D. Lenig, C. R. Mackay, and A. Lanzavecchia. 1998. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J. Exp. Med. 187:875.*
44. Möller, G. 1975. One non-specific signal triggers B lymphocytes. *Transplant Rev. 23.*