Published in final edited form as:
Vaccine. 2008 December 30; 26(Suppl 8): I94–I99. doi:10.1016/j.vaccine.2008.11.062.

Herpes Simplex Virus as a Tool to Define the Role of Complement in the Immune Response to Peripheral Infection

Mark A. Brockman[1] and **David M. Knipe**[2,*]

[1]Partners AIDS Research Center, Massachusetts General Hospital, Boston MA

[2]Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA

Abstract

A complex network of interactions exist between the innate and adaptive immune pathways, which act together to elicit a broad and durable host response following pathogen infection. The importance of the complement system in the host's defense against viruses has become increasingly clear as a result of detailed studies using transgenic mouse models that disrupt specific components of this host immune mechanism. We have utilized herpes simplex virus and replication-defective mutant strains to examine the impact of the complement system on development and maintenance of humoral immune responses. Here we review work from our group and others that highlight the central role that complement proteins C3 and C4 and complement receptors Cr1/Cr2 play during viral infection. We discuss the implications of these results in the context of pathogen infection and current vaccine strategies.

1. Introduction

The major goal of vaccine design is to provide protective immunity in a naïve host, such that the occurrence of infection will result in a dramatically shortened illness to the individual and a significantly reduced disease burden on the population. An effective host immune response to viral infection typically requires the recognition and elimination of pathogen; however, in cases of a chronic viral infection such as HIV or HSV, significant containment of infection in the absence of viral clearance may be sufficient to reduce disease pathology [1,2]. A greater understanding of host immunity in the context of infection with chronic viruses may provide essential clues to their pathogenesis and enable the development of effective vaccines. Recent evidence from a number of groups has demonstrated that innate immune factors can play a key role in enhancing the adaptive immune response to infection. Our work has identified complement proteins C3 and C4 and the complement receptors Cr1 (CD35) and/or Cr2 (CD21) as central mediators of the antibody response following infection with herpes simplex virus (HSV) [3-6]. Durable immunity to replication-defective HSV strains, which may serve as novel vaccine vectors, is similarly dependent on complement activity [3,7] and provide a unique opportunity to examine the role of the complement system during peripheral antigen encounter. In combination with results from other pathogen models, our data suggest that the role of complement during natural pathogen infection should be further investigated and that this innate system can perhaps be harnessed to benefit current vaccine strategies.

*corresponding author: David M. Knipe, PhD., david_knipe@hms.harvard.edu.

2. Innate Immunity and the Complement System

The innate immune system is comprised of specialized cells and molecules that can act in concert to recognize and inhibit viral infection. These include macrophages [8,9], natural killer (NK) cells [10-12], and dendritic cells (DC) [13], cellular receptors such as Toll-like receptors (TLR) and other pattern recognition receptors [14,15], and soluble effector proteins including natural antibody [16,17], interferon and other cytokines [18,19], and the complement family of serum proteins [20,21]. Together, these factors create an extensive 'non-specific' immune network that strives to rapidly neutralize pathogen infection and can also modulate the formation of adaptive immune responses [22].

The complement system includes more than 20 proteins and is activated by antibody-dependent or antibody-independent pathways [23], resulting in a cascade of enzymatic activities. The 'classical pathway' is initiated when the C1 protein [24] encounters IgG, IgM, or mannose-binding protein bound to antigen [25,26]. The classical pathway C3 convertase enzyme (C4b2a) forms and activates the C3 protein by cleaving it into the C3a and C3b fragments. This results in the covalent attachment of C3b to the antigen [27,28] and C3a is released as a soluble product. The 'alternative pathway' is triggered with C3 protein itself becomes activated and attaches non-specifically to nearby antigen [29]. The alternative pathway C3 convertase enzyme (C3bBb) is generated, resulting in a positive-feedback loop and rapid accumulation of activated C3. The C3 protein plays a central role in the complement mechanism. During activation, C3 is cleaved into multiple fragments [30,31], which functions in either the progression of the cascade by forming additional C3 convertase (C3b), the generation of inflammatory responses by recruiting neutrophils, monocytes, and mast cells (C3a) [32,33], or boosting adaptive responses by enhancing antigen trapping (C3b), or by providing a secondary signal to B cells (C3d). Downstream effects of C3 activation include the formation of the C5 convertase complex that is required for generation of the Membrane Attack Complex, composed of the C5, C6, C7, C8, and C9 proteins. This pore-forming structure is responsible for complement-mediated lysis of foreign cells and is also able to directly lyse enveloped viral particles [34].

The identification of C3 receptors on a number of cell types suggests that they may play a key role during establishment of host immunity [35]. Cr1 (CD35) is found on macrophages, B cells, and FDC [36]. It binds to the C3b fragment and mediates adherence, phagocytosis, and antigen localization within the germinal center (GC) [37,38]. Cr2 (CD21) is expressed on B cells, activated T cells, epithelial cells, and FDC, and it binds to the C3d fragment [39-41]. This interaction enhances B cell activation within the GC [42]. Indeed, *Cr2* null mice, which are deficient for Cr1 (CD35) and Cr2 (CD21), demonstrate impaired antigen retention in the spleen and lymph node [43,44], and adoptive transfer experiments have directly linked IgG deficiency with the loss of Cr2 on B cells [45]. The attachment of C3d fragments to antigen provides a co-stimulatory signal to B cells via the Cr2/CD19/CD81 complex [46,47], inducing B cells to proliferate at lower antigen concentrations. As a result, transgenic mice lacking *Cr2*, *CD19*, or *C3* display similar phenotypes [45,48-50]. In addition, it has been reported that fusion of C3d to a protein in one, two, or three copies can magnify the humoral response by 100-, 1,000- and 10,000-fold, respectively [51,52]. Additional C3 receptors, Cr3 (CD11b/CD18) and Cr4 (CD11c/CD18), are present on macrophages, NK cells, neutrophils, T cells, mast cells, and FDC [53], where they have been shown to bind to the C3b fragment. Both Cr3 and Cr4 receptors appear to be involved in phagocytosis of antigen/C3b complexes [54,55] and also have important cell adhesion functions. It has been reported that Cr3 can stimulate macrophages to produce soluble inflammatory factors, such as IL-12 [56], TNF-α [57], and nitric oxide [58], providing additional avenues to enhance adaptive responses.

3. HSV Pathogenesis and Host Immunity

Exposure to HSV typically occurs in the periphery, often at mucosal surfaces or through disrupted skin, and replication within permissive cells at these sites results in visible vesicular lesions [59]. During primary infection, virus encounters the axon termini of innervating sensory nerve cells and travels by retrograde transport to the neuronal cell body [60] where HSV establishes a latent infection within the sensory ganglia [61,62]. During latent infection, the viral genome persists within the neuron, but gene expression is limited to a latency-associated transcript (LAT) [63]. LAT can promote lytic gene silencing [64], at least in part through heterochromatin [65]. Recent studies have shown that viral microRNAs can inhibit viral gene expression [66], and some of these may be derived from LAT. Periodic reactivation from latency results in anterograde transport of virus particles to the axon terminus and re-initiation of infection at or near the site of primary exposure. In rare cases, HSV infection can spread beyond the skin or sensory neuron to involve internal organs or extensive spread within the central nervous system (CNS) [59]. The resulting diseases, including HSV encephalitis, occur most often in neonates or immunocompromised patients and are frequently fatal [67]. HSV infection and reactivation in the eye can result in scarring of the cornea and eventual loss of vision, and HSV keratitis remains a leading cause of blindness in the United States [68]. Gential HSV-2 infection increases the risk of HIV infection [69].

Clearance of the HSV from the periphery requires cell-mediated immune responses, and cytotoxic CD4 and CD8 T cells have been isolated from mice following infection [70,71]. Depletion and adoptive transfer studies have indicated that either CD4 or CD8 T cells alone are sufficient for viral clearance [72], suggesting that a common pathway, such as cytokine expression, may be essential to control HSV infection. In fact, IFN-γ-mediated mechanisms have been proposed to play a critical role during the immune response to lytic HSV infection [72,73]. In small animal models, it has been shown previously that antibody to HSV cannot protect the host from primary infection or lesion formation [74]. However, serum IgG concentration does inversely correlate lethal complications of HSV infection, including CNS disease, in humans [75] and mice [76]. Passive transfer of immune serum has been shown to protect naïve mice from death (i.e. encephalitis) following peripheral HSV infection [77,78], further indicating that antibody can limit spread of virus within the nervous system. Local protection and protection against latent infection requires cellular immunity, however [79].

Antibody has been shown to neutralize HSV by both complement-dependent and - independent mechanisms [80], including virion aggregation, inhibition of viral receptor-mediated fusion, or by blocking post-entry steps such as viral uncoating [81]. In addition, antibody is involved in the opsonization of virus particles, facilitating their uptake by professional APCs, and can activate the classical pathway of the complement system, resulting in complement-mediated lysis of enveloped virus and infected cells. Finally, IgG antibody can redirect NK cells to lyse antibody-bound target cells in a process referred to as antibody-dependent cellular cytotoxicity (ADCC) [82]. In the case of HSV, IgG appears to reduce the spread of virus from the periphery into the nervous system [77,78], and transfer of HSV immune serum to naïve mice up to 24 hours after a lethal inoculation in the footpad can protect animals from death [83,84]. Notably, *in vitro* ADCC results correlate with *in vivo* protection from HSV better than *in vitro* neutralization [85], and passive transfer of F(ab') fragments have been shown to be less protective in mice than complete IgG [86].

4. Role of Complement in Humoral Immunity to HSV

4.1 Antibody responses to HSV infection require complement

In initial studies, we examined the role of complement in the humoral response to HSV following subcutaneous inoculation [3]. Mice deficient in C3 or C4 produced significantly less

IgG antibody following viral infection compared with wild type (wt) mice. A similar lack of humoral immune responses to HSV was observed with *Cr2* null mice, which are deficient in both Cr1 (CD35) and Cr2 (CD21). *C3* null and *Cr2* null mice also failed to develop IgG responses to a heterologous antigen, β-galactosidase (β-gal), expressed by a replication-defective HSV strain. Furthermore, GC failed to develop in *C3* null animals, indicating that there was a severe early defect in B cell activation. Examination of CD4 T cell proliferation to virus or β-gal was similar in mutant and wt mice, demonstrating that deficiencies in these animals were specific to the humoral response. Based on these results, we concluded that enhancement of the antiviral IgG response was mediated by the classical pathway, because it was C4-dependent and required the C3 receptors Cr1 and/or Cr2. It is likely that the defect in IgG responses we observed in the absence of a functional complement system was due to the failure of viral antigens to exceed a natural threshold for B cell activation and survival within the GC. In the absence of C3d/antigen complexes and/or CR1/Cr2 receptor expression, specific B cells did not mature or develop into memory cells. Recent results from the Friedman group have demonstrated that natural IgM antibody acts as a major trigger of the classical complement pathway in response to HSV, resulting in direct neutralization of infectious virus [87]. Therefore, activation of the classical complement pathway appears to play a key role in at least two antiviral immune processes.

4.2 Myeloid cells are necessary to restore humoral responses to peripheral infection in *C3* null mice

Because HSV infection typically occurs at mucosal surfaces and in dermal tissues, we used this physiologically relevant model to examine whether bone marrow-derived cells could provide sufficient complement protein C3 to restore humoral responses to viral antigens following peripheral infection in *C3* null mice. In one study [4], *C3* null mice were reconstituted with bone marrow (BM) from wt mice, and these wt BM→*C3* null chimeric animals were inoculated with HSV by an intradermal route. Our results demonstrated that C3 derived from wt BM cells was sufficient to completely restore humoral responses to peripheral viral infection in the absence of systemic (i.e. liver-derived) C3. The wt BM→*C3* null chimeric animals also displayed C3 mRNA synthesis and protein deposition in lymphoid organs draining the site of infection. In total, these findings indicate that BM-derived cells producing C3 protein are sufficient to enhance of B cell activation and production of virus-specific IgG antibody. Because it has been demonstrated that myeloid/macrophage cells present in BM can produce numerous complement factors, including C3 and C4 proteins [5,88], it is likely that the central mediator of complement activity in this studies was a myeloid-derived cell population. In a series of follow-up experiments [6], we examined whether BM-derived C3 protein was in fact essential for the humoral response to peripheral HSV infection. Using a similar chimeric model system, we reconstituted lethally-irradiated wt mice with BM cells from *C3* null animals, and the resulting C3 null-BM→wt chimeric mice were inoculated with HSV by an intradermal route. Even though the C3 null-BM→wt chimeric animals displayed high levels of C3 protein in the blood and could respond normally to antigen delivered intravenously, they failed to elicit a humoral response following intradermal HSV infection. In fact, IgG titers in the C3 null-BM→wt chimeric mice were comparable to *C3* null control mice, suggesting that serum C3 protein was unable to modulate humoral responses in this peripheral infection model. Cellular infiltration at the site of dermal infection was similar in C3 null-BM→wt chimeric and wt animals; however, only wt mice generated GC in draining lymphoid tissues following infection. These results strongly support a model in which BM-derived cells, presumably of a myeloid/macrophage lineage, are essential sources of complement factors in the dermis, and that these cells play a key role in enhancing humoral immunity to pathogen infections localized to the periphery.

4.3. Optimal long-term humoral responses require complement receptor expression on stromal cells

Because the complement system appeared to be a key factor in the generation of humoral immune responses to peripheral infection, we examined the role that complement plays in maintenance of durable antibody responses following resolution of infection. To address this issue, we utilized replication-defective HSV strains, which we have shown to elicit robust and durable immune responses in wt mice [79,89,90]. They can induce protective immunity against HSV infection [91-93], and they have been modified to produce heterologous antigens to serve as novel vaccine vectors [94,95]. In addition, replication-defective HSV strains provide a unique approach to limit viral antigen and infected cells to a defined time period and peripheral location. To examine the role of complement during late stages of the humoral response, we compared wt and *Cr2* null mice with *Cr2* null animals reconstituted with wt BM [7]. Mice were inoculated three times with a replication-defective HSV strain that also expressed β-gal using an intramuscular route. Peak IgG titers were similar in wt and wt BM→*Cr2* chimeric animals, indicating that BM-derived cells were sufficient to mount robust humoral responses in this system. Peak IgG titers were significantly reduced in *Cr2* null controls, as expected. Following peak responses, wt mice maintained durable IgG titers for greater than 30 weeks post-infection, whereas wt BM→*Cr2* chimeras showed significant decay in serum IgG titers to background levels by week 17. Notably, attempts to boost the wt BM→*Cr2* chimeric animals at week 32 failed to elicit recall responses in these mice, arguing that B cell memory had been lost by this time. These results demonstrate that complement receptor Cr1 and/or Cr2 play a key role in maintaining humoral responses when antigen is limiting. These observations are consistent with a model in which Cr1/Cr2-mediated retention of viral antigen on FDC allows ongoing stimulation of B cells that is necessary to ensure optimal memory cell development and/or maintenance.

4.4. Immune evasion by HSV proteins may enable infection in the presence of pre-existing immunity

HSV encodes a number of potential immune evasion molecules, including some that may directly impact the ability of complement and antibody to neutralize viral infection [96]. The heterodimer of viral glycoproteins E and I (gE/gI) forms an Fc receptor that can bind to human IgG antibody [97]. This activity of gE/gI has been reported to protect the virion from antibody-dependent neutralization and to protect virus-infected cells from ADCC [97]. Also, the viral gC protein binds to the C3 protein and has been shown to protect the virion from complement-mediated neutralization and to protect virus-infected cells from complement-mediated lysis [87,98,99]. We have observed that replication-defective HSV vectors elicit similar IgG responses in the absence or presence of pre-existing host immunity to virus [90]. The inherent ability of HSV to evade innate and adaptive responses (including IgG antibody and complement) may play an important role in natural pathogenesis of this virus in the skin or at mucosal surfaces. Because our results suggest that complement factors may be limited at peripheral sites, even a brief delay in activation of innate immunity might be essential to allow HSV to replicate and to infect a sufficient number of sensory neurons to establish latent infection. This hypothesis is consistent with recent data using HSV strains lacking the gC protein were less virulent in wt mice [99] and that both C3 and antibody appeared to be necessary to control zosteriform disease [100].

5. Role of Complement in Adaptive Responses to Other Viruses

Our results obtained using HSV are largely consistent with reports using other viral pathogens. Complement-dependent enhancement of adaptive host immunity has been observed during numerous viral infections, including vesicular stomatitis virus (VSV) [17], West Nile virus [101] and lymphocytic choriomeningitis virus (LCMV) [102]. In addition, complement

evasion molecules or strategies have been identified in a broad range of viruses, such as other herpesviruses, poxviruses, and HIV [103], strongly suggesting that most viruses must counteract the pressure of complement activity during in vivo replication. Inherent variability between viral pathogens, such as the route of entry and establishment of persistent infection, may result in distinct interactions with innate factors, and these issues should be the focus of future studies. In addition, differences in experimental models, such as inoculum dose, route of infection, and mouse strains used, should be addressed, because these may also play a major role in determining whether complement or other innate factors are necessary for enhancing immunity when antigen concentrations are limited.

6. Implications for vaccine design

Work to understand the role that complement and other innate immune pathways on plays to enhance host immune responses to viral infection may have immediate implications for the field of vaccine design. In the case of HSV, virus can establish a latent infection and reactivate to form lesions at peripheral sites in the skin or mucosa, despite the presence of an ongoing systemic immune response. The ability of HSV to evade innate immune factors like complement and antibody at these peripheral sites may play an important role in this natural course of infection and reactivation. Similar mechanisms are likely essential to allow replication-defective HSV vectors to function well in naïve and pre-immune animals. Indeed, efforts to harness viral immune evasion strategies have already provided unique strategies to overcome pre-existing immunity in the context of viral vaccine vectors [104], and covalent attachment of C3d to antigens has already proven to enhance humoral immune responses following vaccination [51,52]. A better appreciation of mechanisms involved in host immune responses to viral infection, and methods that viruses use to counteract them, may identify essential mediators of vaccine efficacy or result in novel ways to enhance vaccine design.

7. Conclusions

Results from a number of laboratories demonstrate that innate factors can play a key role in enhancing adaptive immune responses to infection and vaccination, particularly when antigen concentrations are low. Our work, as well as that of others, highlights the importance that complement factors produced by BM-derived cells, likely of myeloid lineage, play during the generation of humoral responses to peripheral viral infection. Immune responses initiated in peripheral tissues may be inherently dependent on enhancement by innate immunity, because antigen trapping in the draining lymphoid tissues and lymphocyte activation and trafficking to the site of infection are otherwise inefficient processes when antigen concentrations are limited. Additional studies are needed to further define the mechanisms of complement-mediated activity in the periphery, and we believe that HSV provides an excellent model system to explore these questions. Furthermore, replication-defective mutant strains of HSV may provide novel tools to study aspects of the innate and adaptive immune response following localized infection and antigen presentation at distal sites. A better understanding of innate immune responses and their roles in modulating adaptive immunity, interactions between viral pathogens and innate systems, and mechanisms that viruses and other pathogens use to evade innate factors, will likely contribute important new information to enhance therapies and vaccines intended to protect against human disease.

Acknowledgments

Research in DMK's laboratory on HSV vaccines and vaccine vectors is supported by NIH HIVRAD grant AI46006 and NIH grant AI57552. We thank Michael Carroll and Admar Verschoor for their collaboration in the studies of HSV and complement.

References

1. Letvin NL, Walker BD. Immunopathogenesis and immunotherapy in AIDS virus infections. Nat Med 2003;9:861–6. [PubMed: 12835706]
2. Davenport MP, Ribeiro RM, Chao DL, Perelson AS. Predicting the impact of a nonsterilizing vaccine against human immunodeficiency virus. J Virol 2004;78:11340–51. [PubMed: 15452255]
3. Da Costa XJ, Brockman MA, Alicot E, Ma M, Fischer MB, Zhou X, et al. Humoral response to herpes simplex virus is complement-dependent. Proc Natl Acad Sci USA 1999;96:12708–12. [PubMed: 10535987]
4. Verschoor A, Brockman MA, Knipe DM, Carroll MC. Cutting edge: Myeloid complement c3 enhances the humoral response to peripheral viral infection. J Immunol 2001;167:2446–51. [PubMed: 11509581]
5. Gadjeva M, Verschoor A, Brockman MA, Jezak H, Shen LM, Knipe DM, et al. Macrophage-derived complement component C4 can restore humoral immunity in C4-deficient mice. J Immunol 2002;169:5489–95. [PubMed: 12421924]
6. Verschoor A, Brockman MA, Gadjeva MA, Knipe DM, Carroll MC. Myeloid C3 determines induction of humoral responses to peripheral herpes simplex virus infection. J Immunol 2003;171:5363–71. [PubMed: 14607939]
7. Brockman MA, Verschoor A, Zhu J, Carroll MC, Knipe DM. Optimal Long-Term Humoral Responses to Replication-Defective Herpes Simplex Virus Require CD21/CD35 Complement Receptor Expression on Stromal Cells. J Virol 2006;80:7111–7. [PubMed: 16809316]
8. Heise MT, Virgin HW 4. The T-cell-independent role of gamma interferon and tumor necrosis factor alpha in macrophage activation during murine cytomegalovirus and herpes simplex virus infections. J Virol 1995;69:904–09. [PubMed: 7815559]
9. Sarmiento M. Intrinsic resistance to viral infection. Mouse macrophage restriction of herpes simplex virus replication. J Immunol 1988;141(8):2740–48. [PubMed: 2844905]
10. Orange JS, Biron CA. An absolute and restricted requirement for IL-12 in natural killer cell INF-gamma production and antiviral defense. Studies of natural killer and T cell responses in contrasting viral infections. J Immunol 1996;156:1138–42. [PubMed: 8557990]
11. Rager-Zisman B, Quan PC, Rosner M, Moller JR, Bloom BR. Role of NK cells in protection of mice against herpes simplex virus-1 infection. J Immunol 1987;138:884–88. [PubMed: 3805719]
12. Welsh RM, Dundun PL, Eynon EE, Brubaker JO, Koo GC, O'Donnell CL. Demonstration of the antiviral role of natural killer cells in vivo with a natural killer cell-specific monoclonal antibody (NK1.1). Nat Immun Cell Growth Regulat 1990;9:112–20.
13. Kadowaki N, Antonenko S, Lau JY, Liu YJ. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. Journal of Experimental Medicine 2000;192:219–26. [PubMed: 10899908]
14. van Vliet SJ, den Dunnen J, Gringhuis SI, Geijtenbeek TB, van Kooyk Y. Innate signaling and regulation of Dendritic cell immunity. Curr Opin Immunol 2007;19
15. Geijtenbeek TB, van Vliet SJ, Engering A, 't Hart BA, van Kooyk Y. Self-and nonself-recognition by C-type lectins on dendritic cells. Annu Rev Immunol 2004;22:33–54. [PubMed: 15032573]
16. Baumgarth N, Herman OC, Jager GC, Brown L, Herzenberg LA. Innate and acquired humoral immunities to influenza virus are mediated by distinct arms of the immune system. Proc Natl Acad Sci USA 1999;96:2250–5. [PubMed: 10051627]
17. Ochsenbein AF, Pinschewer DD, Odermatt B, Carroll MC, Hengartner H, Zinkernagel RM. Protective T cell-independent antiviral antibody responses are dependent on complement. J Exp Med 1999;190:1165–74. [PubMed: 10523614]
18. Parr EL, Parr MB. Immune responses and protection against vaginal infection after nasal or vaginal immunization with attenuated herpes simplex virus type-2. Immunology 1999;98:639–45. [PubMed: 10594699]
19. Suzutani T, Nagamine M, Shibaki T, Ogasawara M, Yoshida I, Daikoku T, et al. The role of the UL41 gene of herpes simplex virus type 1 evasion of non-specific host defence mechanisms during primary infection. Journal of General Virology 2000;81:1763–71. [PubMed: 10859382]

20. Anderson DR, Carthy CM, Wilson JE, Yang D, Devine DV, McManus BM. Complement component C3 interactions with coxsackievirus B3 capsid proteins: innate immunity and the rapid formation of splenic antiviral germinal centers. J Virol 1997;71:8841–885. [PubMed: 9343244]
21. Boere WA, Benaissa-Trouw BJ, Harmsen T, Erich T, Kraaijeveld CA, Snippe H. The role of complement in monoclonal antibody-mediated protection against virulent Semliki Forest virus. Immunology 1986;58:553–9. [PubMed: 3015781]
22. Morgan BP, Marchbank KJ, Longhi MP, Harris CL, Gallimore AM. Complement: central to innate immunity and bridging to adaptive responses. Immunol Lett 2005;97:171–9. [PubMed: 15752555]
23. Carroll MC. The complement system in regulation of adaptive immunity. Nat Immunol 2004;5:981–6. [PubMed: 15454921]
24. Heyman B, Pilstrom L, Shulman M. Complement activation is required for IgM-mediated enhancement of the antibody response. J Exp Med 1988;167:1999–2004. [PubMed: 3385360]
25. Boes M, Prodeus A, Schmidt T, Carroll MC, Chen J. A critical role of natural IgM in immediate response against systemic bacterial infection. J Exp Med 1998;188:2381–86. [PubMed: 9858525]
26. Epstein J, Eichbaum QE, Sheriff S, Ezekowitz RAB. The collectins in innate immunity. Curr Opin Immunol 1996;8:29–35. [PubMed: 8729443]
27. Law SK, Lichtenberg NA, Levine RP. Covalent binding and hemolytic activity of complement proteins. Proc Natl Acad Sci 1980;77:7194–98. [PubMed: 6938964]
28. Tack BF, Harrison RA, Janatova J, Thomas ML, Prahl JW. Evidence for presence of an internal thiolester bond in third component of human complement. Proc Natl Acad Sci USA 1980;77:5764–68. [PubMed: 6934510]
29. Devaux P, Christiansen D, Fontaine M, Gerlier D. Control of C3b and C5b deposition by CD46 (membrane cofactor protein) after alternative but not classical complement activation. Eur J Immunol 1999;29:815–22. [PubMed: 10092084]
30. Feldbush TL, Hobbs MV, Severson CD, Ballas ZK, Weiler JM. Role of complement in the immune response. Fed Proc 1984;43:2548–52. [PubMed: 6610570]
31. Fleisher TA, Berger M. Immunoregulatory effects of C3 and its major cleavage fragments. Clin Immunol Immunopathol 1984;33:391–401. [PubMed: 6333950]
32. Daffern PJ, Pfeifer PH, Ember JA, Hugli TE. C3a is a chemotaxin for human eosinophils but not for neutrophils. I. C3a stimulation of neutrophils is secondary to eosinophil activation. J Exp Med 1995;181:2119–27. [PubMed: 7760001]
33. Hartmann K, Henz BM, Krüger-Krasagakes S, Köhl J, Burger R, Guhl S, et al. C3a and C5a stimulate chemotaxis of human mast cells. Blood 1997;89:2863–70. [PubMed: 9108406]
34. Hirsh RL. The complement system. Microbiol Rev 1982;46:71–85. [PubMed: 7045625]
35. Prodinger, WM.; Wurzner, R.; Erdei, A.; Dierich, MP. Complement. In: Paul, WE., editor. Fundamental Immunology. Vol. 4. Philiadelphia: Lippencott-Raven; 1999. p. 967-95.
36. Pepys MB. Role of complement in the induction of immunological responses. Transplantation Reviews 1976;32:93–120. [PubMed: 790690]
37. Brown EJ. The interaction of small oligomers of complement 3B (C3B) with phagocytes. High affinity binding and phorbol ester-induced internalization by polymorphonuclear leukocytes. J Biol Chem 1989;264:6196–201. [PubMed: 2649497]
38. Tew JG, Mandel TE, Miller GA. Immune retention: immunological requirements for maintaining an easily degradable antigen in vivo. Aust J Exp Biol Med Sci 1979;57:401–14. [PubMed: 94545]
39. Clemenza L, Isenman DE. Structure-guided identification of C3d residues essential for its binding to complement receptor 2. J Immunol 2000;165:3839–48. [PubMed: 11034390]
40. Molina H, Kinoshita T, Webster CB, Holers VM. Analysis of C3b/C3d binding sites and factor I cofactor regions within the mouse complement receptors 1 and 2. J Immunol 1994;153:789–95. [PubMed: 8021513]
41. Qin D, Wu J, Carroll MC, Burton GF, Szakal AK, Tew JG. Evidence for an important interaction between a complement-derived CD21 ligand on follicular dendritic cells and CD21 on B cells in the initiation of IgG responses. J Immunol 1998;161:4549–54. [PubMed: 9794381]

42. Mongini PK, Vilensky MA, Highet PF, Inman JK. The affinity threshold for human B cell activation via the antigen receptor complex is reduced upon co-ligation of the antigen receptor with CD21 (CR2). J Immunol 1997;159:3782–91. [PubMed: 9378965]
43. Fang Y, Xu C, Fu YX, Holers VM, Molina H. Expression of complement receptors 1 and 2 on follicular dendritic cells is necessary for the generation of a strong antigen-specific IgG response. J Immunol 1998;160:5273–9. [PubMed: 9605124]
44. Fischer MB, Ma M, Goerg S, Zhou X, Xia J, Finco O, et al. Regulation of the B cell response to T-dependent antigens by classical pathway complement. J Immunol 1996;157:549–56. [PubMed: 8752901]
45. Molina H, Holers VM, Li B, Fung Y, Mariathasan S, Goellner J, et al. Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. Proc Natl Acad Sci USA 1996;93:3357–61. [PubMed: 8622941]
46. Carter RH, Fearon DT. CD19: lowering the threshold for antigen receptor stimulation of B cells. Science 1992;256:105–7. [PubMed: 1373518]
47. Fearon DT. The complement system and adaptive immunity. Semin Immunol 1998;10:355–61. [PubMed: 9799710]
48. Ahearn JM, Fischer MB, Croix DA, Goerg S, Ma M, Xia J, et al. Disruption of the Cr2 locus results in a reduction in B-1a cells and in an impaired B cell response to T-dependent antigen. Immunity 1996;4:251–62. [PubMed: 8624815]
49. Engel P, Zhou LJ, Ord DC, Sato S, Koller B, Tedder TF. Abnormal B lymphocyte development, activation, and differentiation in mice that lack or overexpress the CD19 signal transduction molecule. Immunity 1995;3:39–50. [PubMed: 7542548]
50. Rickert RC, Rajewsky K, Roes J. Impairment of T-cell-dependent B-cell responses and B-1 cell development in CD19-deficient mice. Nature 1995;376:352–5. [PubMed: 7543183]
51. Dempsey PW, Allison MED, Akkaraju S, Goodnow CC, Fearon ST. C3d of complement as a molecular adjuvant: Bridging innate and acquired immunity. Science 1996;271:348–50. [PubMed: 8553069]
52. Toapanta FR, Ross TM. Complement-mediated activation of the adaptive immune responses: role of C3d in linking the innate and adaptive immunity. Immunol Res 2006;36:197–210. [PubMed: 17337780]
53. Wagner C, Hansh GM, Stegmaier S, Denefleh B, Hug F, Schoels M. The complement receptor 3, CR3 (CD11b/CD18), on T lymphocytes: activation-dependent up-regulation and regulatory function. Eur J Immunol 2001;31:1173–80. [PubMed: 11298342]
54. Worth RG, Mayo-Bond L, van de Winkel JG, Todd RFr, Petty HR. CR3 (alphaM beta2; CD11b/CD18) restores IgG-dependent pahgocytosis in transfectants expressing a phagocytosis-defective Fc gammaRIIA (CD32) tail-minus mutant. J Immunol 1996;157(12)
55. Yan J, Vetvicka V, Xia Y, Hanikyrova M, Mayadas TN, Ross GD. Critical role of Kuffer cell CR3 (CD11b/CD18) in the clearance of IgM-opsonized erythrocytes or soluble beta-glucan. Immunopharmacology 2000;46:39–54. [PubMed: 10665778]
56. Marth T, Kelsall BL. Regulation of interleukin-12 by complement receptor 3 signaling. J Exp Med 1997;185:1987–95. [PubMed: 9166428]
57. van der Laan LJ, Ruuls SR, Weber KS, Lodder IJ, Dopp EA, Dijkstra CD. Macrophage phagocytosis of myelin in vitro determined by flow cytometry: phagocytosis is mediated by CR3 and induces production of tumor necorsis factor-alpha and nitric oxide. J Neuroimmunol 1996;70:145–52. [PubMed: 8898723]
58. Goodrum KJ, McCormick LL, Schneider B. Group B streptococcus-induced nitric oxide production in murine macrophages is CR3 (CD11b/CD18) dependent. Infect Immun 1994;62:3102–7. [PubMed: 8039877]
59. Roizman, B.; Knipe, DM.; Whitley, RJ. Herpes Simplex Virus. In: Knipe, DM.; Howley, PM., editors. Fields Virology. Vol. 5. Philadelphia, Lippincott: Williams and Wilkins; 2007. p. 2501-602.
60. Kristensson K, Lycke E, Roytta M, Svennerholm B, Vahlne A. Neuritic transport of herpes simplex virus in rat sensory neurons in vitro. Effects of substances interacting with microtubular function and axonal flow [nocodazole, taxol and erythro-9-3-(2-hydroxynonyl)adenine]. Journal of General Virology 1986;67:2023–28. [PubMed: 2427647]

61. Klein RJ, Friedman-Kien AE, Brady E. Latent herpes simplex virus in ganglia of mice after primary infection and reinoculation at a distant site. Archives of Virology 1978;57:161–66. [PubMed: 208489]
62. Price RW, Waltz MA, Wohlenberg C, Notkins AL. Latent infection of sensory ganglia with herpes simplex virus: Efficacy of immunication. Science 1975;188:938–40. [PubMed: 166432]
63. Stevens JG, Cook ML. Latent herpes simplex virus in spinal ganglia of mice. Science 1971;173:843–45. [PubMed: 4328483]
64. Garber DA, Schaffer PA, Knipe DM. A LAT-associated function reduces productive-cycle gene expression during acute infection of murine sensory neurons with herpes simplex virus type 1. J Virol 1997;71:5885–93. [PubMed: 9223478]
65. Wang Q-Y, Zhou C, Johnson KE, Colgrove RC, Coen DM, Knipe DM. Herpesviral latency-associated transcript gene promotes assembly of heterochromatin on viral lytic-gene promoters in latent infection. Proc Natl Acad of Sci USA 2005;102:16055–59. [PubMed: 16247011]
66. Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature 2008;454:780–3. [PubMed: 18596690]
67. Olson LC, Buescher EL, Artenstein MS. Herpesvirus infections of the human central nervous system. N Engl J Med 1967;277:1271–77. [PubMed: 4294512]
68. Pepose JS, Keadle TL, Morrison LA. Ocular herpes simplex: changing epidemiology, emerging disease patterns, and the potential of vaccine prevention and therapy. Am J Ophthalmol 2006;141 (3):547–57. [PubMed: 16490506]
69. Wald A, Link K. Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis. J Infect Dis 2002;185:45–52. [PubMed: 11756980]
70. Manickan E, Francotte M, Kuklin N, Dewerchin M, Molitor C, Gheysen D, et al. Vaccination with recombinant vaccinia viruses expressing ICP27 induces protecting immunity against herpes simplex virus through CD4+ Th1+ T cells. J Virol 1995;69:4711–16. [PubMed: 7609036]
71. Yasukawa M, Inatsuki A, Kobayashi Y. Helper activity in antigen-specific antibody production mediated by CD4+ human cytotoxic T cell clones directed against herpes simplex virus. J Immunol 1988;140:3419–25. [PubMed: 2452188]
72. Smith PM, Wolcott RM, Chervenack R, Jennings SR. Control of acute cutaneous herpes simplex virus infection: T cell-mediated viral clearance is dependent upon interferon-gamma. Virology 1994;202(1):76–88. [PubMed: 7912023]
73. Bouley DM, Kanangut S, Wire WS, Rouse BT. Characterization of herpes simplex virus type-1 infection and herpetic stromal keratitis development in IFN-gamma knockout mice. J Immunol 1995;155:3964–741. [PubMed: 7561104]
74. Hayashida I, Nagafuchi S, Hayashi Y, Kino Y, Mori R, Oda H, et al. Mechanism of antibody-mediated protection against herpes simplex virus infection in athymic nude mice: requirement of Fc portion of antibody. Microbiology & Immunology 1982;26:497–509. [PubMed: 6290850]
75. Ashley RL, Dalessio J, Burchett S, Brown Z, Berry S, Mohan K, et al. Herpes simplex virus-2 (HSV-2) type-specific antibody correlates of protection in infants exposed to HSV-2 at birth. Journal of Clinical Investigation 1992;90:511–14. [PubMed: 1322941]
76. Whitley RJ. Neonatal herpes simplex virus infections: is there a role for immunoglobulin in disease prevention and therapy? Pediatric Infectious Disease 1994;13:432–38.
77. McKendall RR. Delayed IgG-mediated clearance of herpes simplex virus type 1 from the CNS but not footpad during the early stages of infection: possible result of relative integrity of the blood-brain barrier. Journal of General Virology 1983;64:1965–72. [PubMed: 6310036]
78. Oakes JE, Lausch RN. Role of Fc fragments in antibody-mediated recovery from ocular and subcutaneous herpes simplex virus infections. Infection & Immunity 1981;33:109–14. [PubMed: 6266961]
79. Morrison LA, Knipe DM. Immunization with replication-defective mutants of herpes simplex virus type 1: Sites of immune intervention in pathogenesis of challenge virus infection. J Virol 1994;68:689–96. [PubMed: 8289372]

80. Spenlehauer C, Kirn A, Aubertin AM, Moog C. Antibody-mediated neutralization of primary human immunodeficiency virus type 1 isolates: investigation of the mechanism of inhibition. J Virol 2001;75:2235–45. [PubMed: 11160727]
81. Edwards MJ, Dimmock NJ. Two influenza A virus-specific Fabs neutralize by inhibiting virus attachment to target cells, while neutralization by their IgGs is complex and occurs simultaneously through fusion inhibition and attachment inhibition. Virology 2000;278:423–35. [PubMed: 11118365]
82. Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol 2001;19:197–223. [PubMed: 11244035]
83. Lubinski J, Nagashunmugam T, Friedman HM. Viral interference with antibody and complement. Semin Cell Dev Biol 1998;9:329–37. [PubMed: 9665870]
84. Sanna PP, DeLogu A, Williamson RA, Hom YL, Strauss SE, Bloom FE, et al. Protection of nude mice by passive immunization with a type-common human recombinant monoclonal antibody against HSV. Virology 1996;215:101–6. [PubMed: 8553581]
85. Mester JC, Glorioso JC, Rouse BT. Protection against zosteriform spread of herpes simplex virus by monoclonal antibodies. Journal of Infectious Diseases 1991;163:263–69. [PubMed: 1846388]
86. McKendall RR. IgG-mediated viral clearance in experimental infection with herpes simplex virus type 1: role for neutralization and Fc-dependent functions but not C' cytolysis and C5 chemotaxis. Journal of Infectious Diseases 1985;151:464–70. [PubMed: 2982965]
87. Hook LM, Lubinski JM, Jiang M, Pangburn MK, Friedman HM. Herpes simplex virus type 1 and 2 glycoprotein C prevents complement-mediated neutralization induced by natural immunoglobulin m antibody. J Virol 2006;80:4038–46. [PubMed: 16571820]
88. Hartung HP, Hadding U. Synthesis of complement by macrophages and modulation of their functions through complement activation. Springer Semin Immunopathol 1983;6:283–326. [PubMed: 6364428]
89. Brubaker JO, Thompson CM, Morrison LA, Knipe DM, Siber GB, Finberg RW. Th1-associated immune responses to beta-galactosidase expressed by replication-defective herpes simplex virus. J Immunol 1996;157:1598–604. [PubMed: 8759744]
90. Brockman M, Knipe DM. Herpes simplex virus vectors elicit a durable antibody response in mice despite the presence of preexisting host immunity. J Virol 2002;76:3678–87. [PubMed: 11907207]
91. Da Costa XJ, Kramer MF, Zhu J, Brockman MA, Knipe DM. Construction, phenotypic analysis, and immunogenicity of a UL5/UL29 double deletion mutant of herpes simplex virus 2. J Virol 2000;74:7963–71. [PubMed: 10933704]
92. Dudek T, Knipe DM. Replication-defective viruses as vaccines and vaccine vectors. Virology 2006;344:230–39. [PubMed: 16364753]
93. Hoshino Y, Pesnicak L, Dowdell KC, Lacayo J, Dudek T, Knipe DM, et al. Comparison of immunogenicity and protective efficacy of genital herpes vaccine candidates herpes simplex virus 2 dl5-29 and dl5-29-41L in mice and guinea pigs. Vaccine 2008;26:4034–40. [PubMed: 18565628]
94. Murphy CG, Lucas WT, Means R, Czajak S, Hale CL, Lifson JD, et al. Vaccine protection against simian immunodeficiency virus by recombinant strains of herpes simplex virus. J Virol 2000;74:7745–53. [PubMed: 10933680]
95. Kaur A, Sanford HB, Garry D, Lang S, Klumpp SA, Watanabe D, et al. Ability of herpes simplex virus vectors to boost immune responses to DNA vectors and to protect against challenge by simian immunodeficiency virus. Virology 2007;357(2):199–214. [PubMed: 16962628]
96. Koelle DM, Corey L. Herpes simplex: insights on pathogenesis and possible vaccines. Annu Rev Med 2008;59:381–95. [PubMed: 18186706]
97. Nagashunmugam T, Lubinski J, Wang L, Goldstein LT, Weeks BS, Sundaresan P, et al. In vivo immune evasion mediated by the herpes simplex virus type 1 immunoglobulin G Fc receptor. J Virol 1998;72:5351–59. [PubMed: 9620988]
98. Friedman HM, Wang L, Fishman NO, Lambris JD, Eisenberg RJ, Cohen GH, et al. Immune evasion properties of herpes simplex virus type 1 glycoprotein gC. J Virol 1996;70:4253–60. [PubMed: 8676446]

99. Lubinski JM, Wang L, Soulika AM, Burger R, Wetsel RA, Colten H, et al. Herpes simplex virus type 1 glycoprotein gC mediates immune evasion in vivo. J Virol 1998;72:8257–63. [PubMed: 9733869]

100. Lubinski JM, Jiang M, Hook L, Chang Y, Sarver C, Mastellos D, et al. Herpes simplex virustype 1 evades theeffects of antibody and complement in vivo. J Virol 2002;76:9232–41. [PubMed: 12186907]

101. Mehlhop E, Diamond MS. Protective immune responses against West Nile virus are primed by distinct complement activation pathways. J Exp Med 2006;203:1371–81. [PubMed: 16651386]

102. Suresh M, Molina H, Salvato MS, Mastellos D, Lambris JD, Sandor M. Complement component 3 is required for optimal expansion of CD8 T cells during a systemic viral infection. J Immunol 2003;170:788–94. [PubMed: 12517942]

103. Lambris JD, Ricklin D, Geisbrecht BV. Complement evasion by human pathogens. Nat Rev Microbiol 2008;6:132–42. [PubMed: 18197169]

104. Schauber-Plewa C, Simmons A, Tuerk MJ, Pacheco CD, Veres G. Complement regulatory proteins are incorporated into lentiviral vectors and protect particles against complement inactivation. Gene Ther 2005;12:238–45. [PubMed: 15550926]