JOURNAL OF VIROLOGY, Nov. 1997, p. 8841–8845 Vol. 71, No. 11
0022-538X/97/$04.00+0

NOTES

Complement Component 3 Interactions with Coxsackievirus B3 Capsid Proteins: Innate Immunity and the Rapid Formation of Splenic Antiviral Germinal Centers

DANIEL R. ANDERSON,[1,2] CHRISTOPHER M. CARTHY,[1] JANET E. WILSON,[1] DECHENG YANG,[1] DANA V. DEVINE,[1] AND BRUCE M. MCMANUS[1]*

Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada,[1] and Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska[2]

Received 20 March 1997/Accepted 6 August 1997

Innate immunity is central to the clearance of pathogens from hosts as well as to the definition of acquired immune responses (D. T. Fearon, and R. M. Locksley, Science 272:50–53, 1996). Coxsackievirus B3 (CVB3), a human cardiopathic virus, was evaluated for the ability to activate the alternative and classical pathway of complement. CVB3 proteins interact with complement component 3 (C3, a soluble protein effector of innate immunity) after either in vitro exposure to mouse serum or in vivo murine infection and activate the alternative pathway of complement. In addition, we demonstrate that viral antigen retention and localization in germinal centers is dependent on C3, while virus antigen retention in extrafollicular regions in the spleen is not. In vivo depletion of native C3 abolished the rapid formation of virus-specific germinal centers (by day 3 post-CVB3 infection) in the absence of serum anti-CVB3 antibodies. These studies demonstrate that innate immune mechanisms, such as C3 interaction with CVB3, are essential for splenic antiviral germinal center formation in naive (antigen nonsensitized) mice resistant (C57BL/6J strain) and susceptible (A/J strain) to CVB3-induced myocarditis.

Coxsackievirus B3 (CVB3), the most prevalent human cardiopathic virus (17), is an enterovirus in the family *Picornaviradae*. Recently, CVB3 has been shown to directly associate with and infect cells of immune origin both in vivo and in vitro (2, 19, 42, 43). Whether this association is mainly adaptive for the host, resulting in antigenic retention and stimulation of the immune response, or mainly pathologic, resulting in disruption of immune regulation, is not known.

The mechanisms of antigen retention for stimulation of immune responses in lymphoid organs have been studied extensively (21, 36, 37, 40, 41). Antigens interact with antibodies and activated complement component 3 (C3) of the alternative complement pathway, and C3b and its cleavage products, C3bi and C3dg, interact in turn with C3 receptors on follicular dendritic cells and B cells. These molecular and cellular interactions play a crucial role in the retention of antigen in lymphoid organs, the formation of immune complex-coated spherical bodies (iccosomes), and the stimulation of germinal centers (36, 37, 40, 41). C3-coated particles are known to be stimulators of antibody-independent, humoral, and cellular immune responses (1, 16). Indeed, recently it has been demonstrated with recombinant protein expression that just three molecules of C3d linked to a thymus-dependent antigen increase the potency of stimulation of an acquired humoral immune response by 10,000-fold (13). Interactions between C3 and infectious agents as well are important in the clearance and inactivation of viral pathogens (5, 18); however, these interactions can also facilitate enveloped virus infections (10) either in an antibody-dependent (8) or antibody-independent (6, 7) fashion.

Virus interactions with the immune system have become an essential area of study in virus research (2, 19, 28, 30, 42, 43). A general paradigm based on the universality of virus interactions with the immune system and immune compartments like the spleen has emerged and reflects a critical balance between host and viral adaptability.

Complement proteins have been shown to be important for enveloped viruses, specifically with regard to herpesviruses and human immunodeficiency virus type 1 (HIV-1). It has been shown that C3 interacts directly with HIV-1, and this interaction is hypothesized to occur with glycosylated viral proteins (29, 32). Furthermore, complement receptors have been shown to be important in the process of HIV-1 infection of cell lines that express complement receptors (6, 7). Thus, complement interactions with infectious agents may regulate the induction, progression, and pathogenesis of virus-induced diseases. We therefore have extended previous studies (2) and have evaluated potential mechanisms of CVB3 localization to germinal centers and extrafollicular regions of the spleen in a murine model of CVB3-induced disease.

Myocarditis-susceptible A/J mice and myocarditis-resistant C57BL/6J mice (Jackson Laboratories) were injected intraperitoneally (i.p.) with cobra venom factor (CVF) (Quidel Corporation) to deplete the serum of C3. A/J and C57BL/6J mice were divided randomly into three groups as follows: (i) animals injected i.p. with 10^5 PFU of CsCl-purified CVB3 and CVF (CVF, $n = 3$ or 4/group), (ii) animals injected with 10^5

* Corresponding author. Present address: Cardiovascular Research Laboratory, McDonald Research Wing, St. Paul's Hospital, 1081 Burrard St., Vancouver, B.C., Canada V6Z 1Y6. Phone: (604) 631-5200. Fax: (604) 631-5208. E-mail: mcmanus@unixg.ubc.ca.

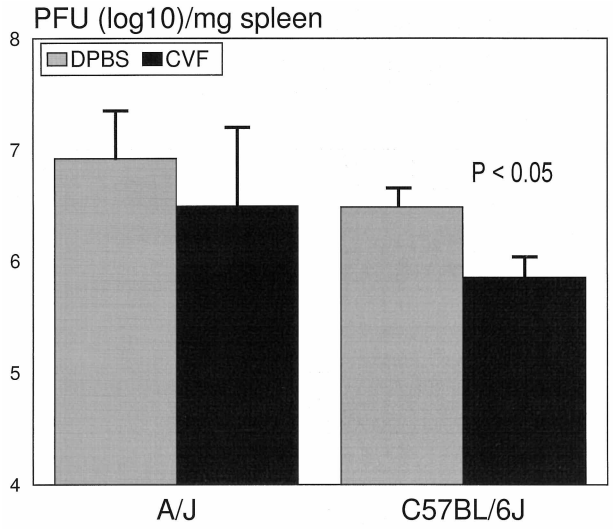

FIG. 1. Load of infectious CVB3 in the spleens of CVB3-infected A/J (n = 3 or 4/group) and C57BL/6J (n = 3/group) mice treated with CVF or DPBS was evaluated at day 3 p.i. There was no significant difference in virus load in the spleens of A/J mice; however, the concentration in the spleens of CVF-treated C57BL/6J mice was statistically lower than that of DPBS-treated animals. Bars, 1 standard deviation (SD).

PFU of CsCl-purified CVB3 and Dulbecco's phosphate-buffered saline (DPBS) (CVF sham, n = 3/group), and (iii) animals injected with CVF and DPBS (CVB3 sham, n = 3/group). Twenty-four hours prior to infection with CVB3 at day 0, groups were injected i.p. with 4 units of CVF/mouse, twice daily for 3 days. Sham animals were injected with DPBS. Depletion of serum C3 in vivo was verified by crossed immunoelectrophoresis (CIE) as discussed in detail below. It should be noted that mice tolerate CVF doses which are 250 to 1,000 times greater than the dose given in this experiment without changes in body weight, spleen weight, splenic histology, plasma volume, or erythrocyte volume in the intravascular or splenic compartments (31).

The titers of CVB3 in tissues were determined on monolayers of HeLa cells by agar overlay plaque assay, as previously described (2). In C3-depleted A/J mice the total load (PFU/mg) of infectious virus in the spleen at day 3 postinfection (p.i.) was no different from that in non-C3-depleted control A/J mice (Fig. 1). In C57BL/6J mice there was a significant decrease in the load (PFU/mg) of CVB3 in the spleen of CVF-treated animals ($P < 0.05$) (Fig. 1). The significant difference in splenic load (PFU/mg) after CVF treatment of C57BL/6J mice may reflect a more marked deficiency in retention of infectious virus in or on cellular fractions of the C57BL/6J spleen. This marked decrease in splenic viral load of myocarditis-resistant mice after CVF treatment may reflect the generally beneficial effect of retention of virus in lymphoid compartments afforded by interaction with complement. While the existence of this protective mechanism is not certain, information derived in separate experiments indicates that when complement depletion occurs, viral titers in heart muscle rise (data not shown).

In situ hybridization (ISH) for sense strand viral RNA was performed as previously described (2, 9, 19). Importantly, ISH positivity for CVB3 genome in spleens of CVF-treated A/J and C57BL/6J animals was dramatically decreased compared to that in spleens of DPBS-treated animals (Ps < 0.001 for the A/J and the C57BL/6J mice) (Fig. 2). There was virtually no hybridization in germinal centers of CVF-treated animals, but there was an increase in extrafollicular signals (Fig. 2). Although the average extrafollicular staining increased in CVF-treated C57BL/6J mice, this increase was not significant. There was, however, a significant increase in extrafollicular staining in A/J mice treated with CVF ($P < 0.01$).

Despite such changes in CVB3 viral load (Fig. 1) and RNA distribution (Fig. 2) after CVB3 depletion, there was no significant difference between the splenic loads (PFU/mg) of CVB3 between A/J and C57BL/6J mice treated with CVF and those between A/J and C57BL/6J mice which were treated with DPBS; these data corroborate the results of previously reported studies (2). Similarly, more virus was in the noncellular fraction (plasma of the spleen) than in the cellular fraction (splenocytes) of the spleen (2). Thus, the quantity of virus as determined by ISH contributes only a fraction to splenic load (PFU/mg) and may be altered significantly without dramatically affecting total splenic load (PFU/mg).

As discussed above, viral loads in the spleen may be altered with CVF treatment (Fig. 1); however, it is also likely that these changes are a reflection of alterations in immune mechanisms secondary to C3 depletion. For example, just as there is a C3-independent increase in extrafollicular localization of CVB3 in the spleens of A/J mice (a hypothesized increase in phagocytosis by marginal zone phagocytic cells), there are likely other alterations in innate immune responses in other reticuloendothelial organs (i.e., an increased phagocytosis by hepatic Kupffer cells). The temporal and systemic effect of C3 depletion on the viral load in host tissues, end organ pathology, and host mortality, remains to be determined.

The splenic ISH pattern of staining in all A/J and C57BL/6J mice treated with CVF was dramatically altered (Fig. 3) and clearly indicates that C3 has a central role in localization of virus to splenic germinal centers after CVB3 infection. The inability to detect CVB3-neutralizing antibodies at day 3 of this

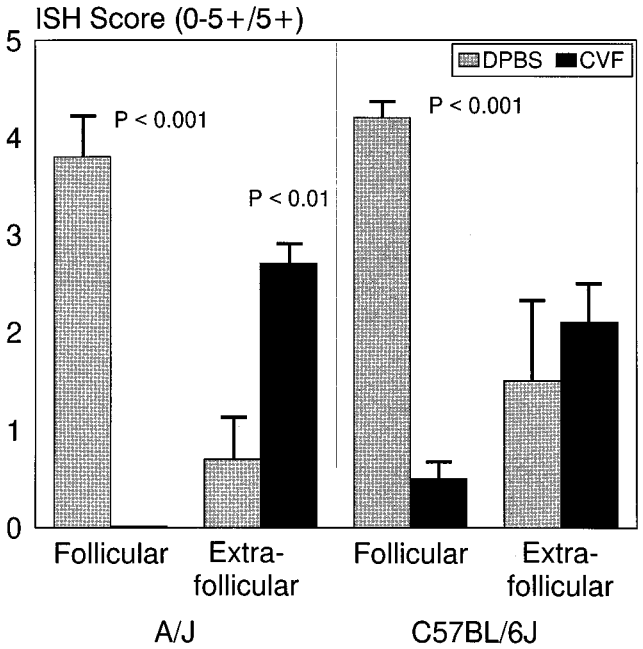

FIG. 2. ISH staining scores are shown for spleens of CVB3-infected A/J and C57BL/6J mice treated with CVF or DPBS and evaluated at day 3 p.i. ISH positivity in A/J and C57BL/6J splenic follicles decreased dramatically after treatment with CVF ($P < 0.001$). Although extrafollicular staining increased after CVF treatment, this increase was significant compared to staining for control hybridization only in A/J mice. Bars, 1 SD.

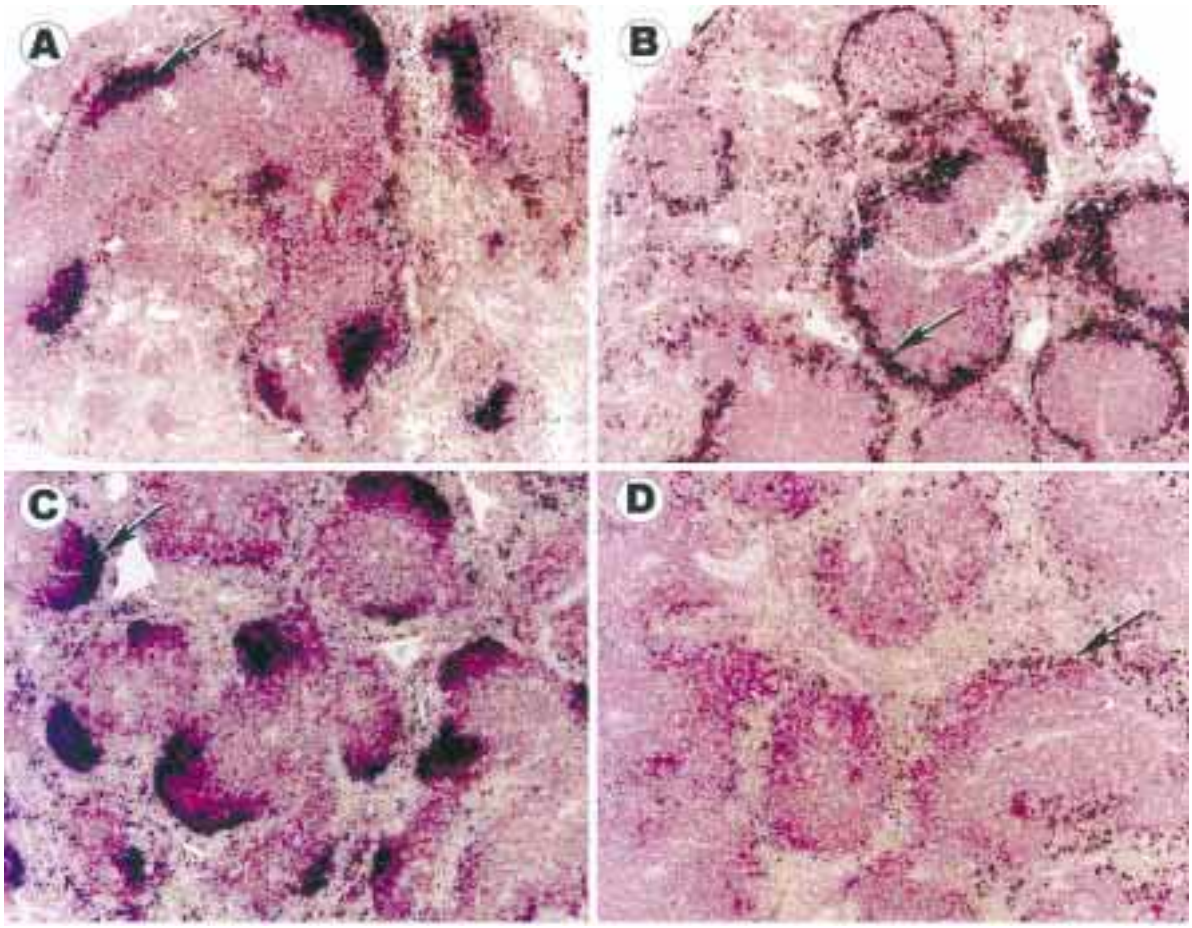

FIG. 3. ISH staining (carmalum counterstain) for the sense strand RNA in the spleens of CVB3-infected A/J (A and B) and C57BL/6J (C and D) mice treated with CVF or DPBS and harvested at day 3 p.i. DPBS treatment (A and C) and CVF treatment (B and D) were strikingly different regarding localization of the virus. The germinal center in panels A and C (arrows) is absent from panels B and D; however, ISH staining is still present in the extrafollicular regions of the CVF-treated spleens (arrows) (B and D). Magnification, ×125.

experiment (data not shown) and at day 4 of previous experiments (data not shown) as well as the absence of anti-CVB3 antibodies (neutralizing and nonneutralizing) at day 4 in experiments performed by others (25, 44) underscore the importance of innate immune responses, specifically C3, in the development and retention of virus in germinal centers and extrafollicular regions by day 3 post-CVB3 infection.

To determine whether CsCl-purified CVB3 particles are able to activate C3 and be fixed by complement proteins, serum-exposed CVB3 particles were assessed for functional complement activity by a hemolytic screening assay as previously described (Fig. 4) (15). No change in functional complement activity was detected, suggesting that there is little or no activation of the classical pathway and modest depletion of C3. Notably, C3 is not a limiting component in this hemolytic assay.

A more sensitive technique, CIE, was employed to evaluate depletion of serum C3 after incubation with CsCl-purified CVB3 as previously described (15). Mouse serum was diluted 1:4 in veronal-buffered saline (VBS) (150 mM NaCl–75 mM sodium barbital [pH 7.5]) and then incubated (30 min at 37°C) with or without CsCl-purified CVB3 as described in the legend to Fig. 4. Serum exposed to CVF was used as a positive control for the conversion of C3 to C3 degradation products, and untreated serum was also tested to verify antibody reactivity (data not shown). Multiple CIE experiments demonstrated that the CsCl-purified CVB3 preparation does interact with C3, resulting in depletion and conversion to C3 cleavage products (Fig. 5). Incubation of purified CVB3 resulted in a 56% reduction of native C3 relative to nondepleted control serum (Fig. 5).

Thus, in the presence of serum, CVB3 capsid proteins (Vp1, Vp2, and Vp3) interact with native C3 to form C3 cleavage products (C3b, C3bi, or C3dg). The rapid development of mature germinal centers by day 3 post-CVB3 infection indicates that antibody-independent processes stimulate germinal center formation within hours of infection, since it takes 3 days for a germinal center to fully develop after antigen exposure (22, 24, 26). It should be noted that in attempts to evaluate the effect of CVF depletion of C3 at later days p.i. (i.e., days 5 to 7), there is significant mortality in both strains of mice with C3 depletion. This result further emphasizes the importance of innate immune responses in the development of end organ damage and animal mortality.

These data (Fig. 3), in the context of previous data (2), as demonstrated by ISH showing the trapping of CVB3 in germinal centers, suggest that B-cell localization is not the consequence of direct C3 fixation of CVB3 and binding to C3 receptors on B cells. Rather, the bulk of this localization is due to the C3-dependent development of CVB3-specific germinal

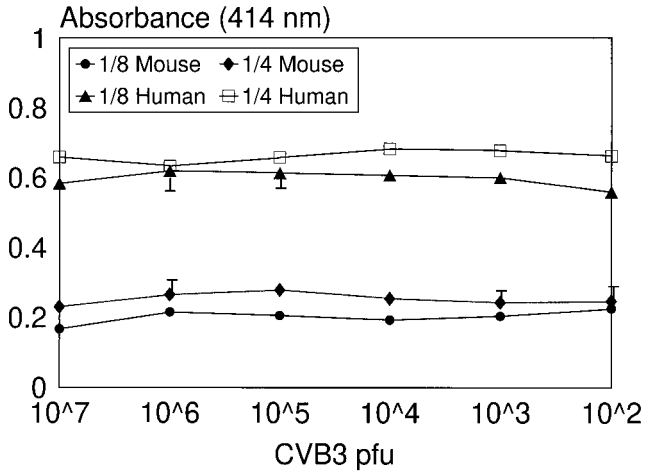

FIG. 4. Absorbance was plotted at 414 nm to evaluate the lysis of sheep erythrocytes after exposure to serum preincubated with 10^7, 10^6, 10^5, 10^4, 10^3, or 10^2 PFU of cesium chloride-purified CVB3. One hundred microliters of CVB3 dilutions (10^7 to 10^2 PFU) in VBS (150 mM NaCl–75 mM sodium barbital [pH 7.5]) was incubated (for 30 min at 37°C) with 100 μl of human or mouse serum diluted 1:4 or 1:8 in $DGVB^{2+}$ (VBS containing 0.1% dextrose–0.1% gelatin–0.5 mM $MgCl_2$–0.15 mM $CaCl_2$), and 300 μl of ice-cold $DGVB^{2+}$ was then added. A hemolytic assay was used to determine the remaining complement activity. Antibody-coated sheep erythrocytes were used as target cells for complement-mediated lysis. Unlysed erythrocytes were removed by centrifugation, and the percentage of cells lysed was determined by measuring hemoglobin release at a wavelength of 414 nm. Lysis of the target cell is directly correlated with the level of residual active classical complement pathway in the serum after exposure to CVB3. A lowered level of active complement after CVB3 exposure was interpreted as evidence of complement consumption during the incubation of serum and CVB3. Human and mouse sera diluted 1/4 and 1/8 showed no changes in absorbance after incubation with different titers of CVB3. The absence of SD bars for certain data points reflects the narrow range of variability and the fact that the deviation bars would be superimposed on each respective data point.

centers. Importantly, formation of iccosomes occurs on some follicular dendritic cells (FDCs) as early as 1 day after secondary antigen challenge (34, 38, 40). When the FDC network has reached its peak size (by day 3 postsecondary antigen challenge) (35, 36), iccosomes have been released from FDC, endocytosed by germinal center B cells, and processed, and antigen has been presented to T cells for cognate help (21). Presentation of antigen by B cells to antigen-specific $CD4^+$ T cells within the FDC network results in the further activation and proliferation of B cells and the maturation of oligoclonal germinal centers by 72 h postantigenic (infection) challenge (24, 26). It is the mature germinal center which is able to retain antigen on surface immunoglobulin-positive B cells (the dark zone of a germinal center) to undergo positive selection and maturation, and it is this retention which is largely reflected in the striking ISH positivity we have observed in spleens of CVB3-infected animals. Thus, the presence of mature germinal centers at day 3 p.i. (2) further suggests that CVB3 is able to initiate the germinal center response within hours of infection; however, in contrast to the above-described models (34, 38, 40), our model is a primary antigenic challenge model. It is during the viremic phase of CVB3 infection (the first few days p.i.) that antigens of intact viral particles are exposed to and "fixed" by serum C3, with subsequent direct stimulation of germinal center formation prior to the synthesis of antiviral antibodies. Furthermore, the nature of the CVB3 particle is likely central to the ability to be "fixed" by C3. Alterations in capsid protein amino acids, which may serve as C3 nucleophiles (i.e., amino or sulfur-containing side chains), can potentially alter C3 and CVB3 interactions and the ensuing disease process. Interestingly, mutations in amino acids in immunodominant or antigenic portions of enteroviral capsid proteins which potentially interact with C3 have indeed affected receptor binding (23) and viral pathogenicity (20). The consequences of C3 and CVB3 interactions are profound not only as a potential mechanism of tissue pathogenesis but also because of their involvement in antigenic clearance and virus-specific immune sensitization (i.e., the phenotypic and functional patterns of immune cells).

As previously mentioned, CVB3 is able to bind to, infect, and replicate in immune cells in vitro and in vivo (2, 19, 42, 43). The mechanisms of such binding, however, have been speculative, since multiple receptors or binding proteins are known to exist. The foregoing data suggest that in addition to the coxsackievirus and adenovirus receptor (3, 29) and previously proposed surface binding proteins (4, 12, 14, 27, 33), CVB3 may be binding to C3 binding proteins such as complement receptors. Tomko et al. (39) did not find murine coxsackievirus and adenovirus receptor expression in the spleens of mice even though studies have shown CVB3 binding and infection of immune cells (2, 19, 42, 43). It is intriguing to speculate that decay accelerating factor, a molecule previously demonstrated to bind CVB3 (4, 33) may bind CVB3 through C3b. This hypothesis follows from data demonstrating the function of decay accelerating factor, namely inactivation of the C3 convertase enzyme by binding C3 cleavage products bound to a "fixed" surface (11).

Finally, the data presented here demonstrate that (i) virus antigen retention in germinal centers is dependent on C3, (ii) virus antigen retention in extrafollicular regions of the spleen is not dependent on C3, and (iii) CVB3 is not a detectable activator of the classical pathway of complement activation but does activate the alternative complement pathway. These virus-complement interactions are essential to germinal center

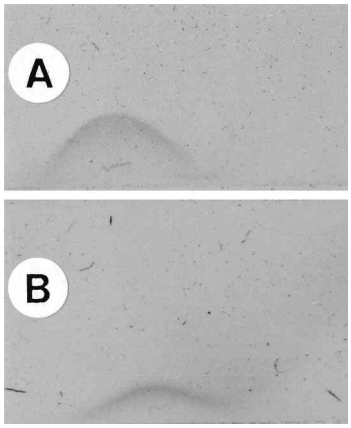

FIG. 5. CIE gels of diluted neat murine serum (A) or murine serum incubated with 10^7 PFU of CsCl-purified CVB3 (B). First-dimension electrophoresis (1% agarose gel at 10 V/cm) was followed by second-dimension electrophoresis (at 2 V/cm overnight) in a 1% agarose gel containing goat anti-mouse C3 (Organon Teknika, Durham, N.C.). Unprecipitated proteins were removed, and gel was stained with Coomassie blue R-250. In CIE, first-dimension electrophoresis separates the proteins by size; thus, C3b migrates further than C3. The second-dimension electrophoresis results in the migration of C3 and C3b, reflecting the precipitin arc. For these experiments, all first-dimension electrophoresis was carried out in the same gel to allow direct comparison among samples. C3 in murine serum incubated with purified CVB3 was partially converted to C3 degradation products. The 56% decrease in the area under the C3 precipitin arc after serum incubation with virus (B) compared to serum incubation alone (A) demonstrates C3 depletion and conversion to C3 cleavage products.

formation early post-CVB3 infection before the detection of circulating antiviral antibodies. Furthermore, these interactions may limit virus load early after infection (within hours p.i.) through splenic immune retention and sequestration in an antibody-independent fashion. These interactions may ultimately limit and/or modify disease and immune processes and mortality during the later stages of infection.

We appreciate deeply the technical support of Lubos Bohunek and the assistance and consultation of Amanda Bradley and Katalin Zay with regard to the hemolytic and CIE assays. We also recognize the fine editorial and photographic contributions of Shelley Wood and Stuart Green, respectively.

This study has been supported by the American Heart Association, the British Columbia Health Research Foundation (B.M.M.), the Heart and Stroke Foundation of British Columbia and Yukon (B.M.M. and C.M.C.), and the Medical Research Council of Canada (D.V.D.) (grant MT-10933) and by a fellowship from the University of Nebraska Alumni Association and the M.D./Ph.D. program of the Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha (D.R.A.).

REFERENCES

1. **Ahmed, R., and D. Gray.** 1996. Immunological memory and protective immunity: understanding their relation. Science **272:**54–60.
2. **Anderson, D. R., J. E. Wilson, C. M. Carthy, D. Yang, R. Kandolf, and B. M. McManus.** 1996. Direct interactions of coxsackievirus B3 with immune cells in the splenic compartment of mice susceptible or resistant to myocarditis. J. Virol. **70:**4632–4645.
3. **Bergelson, J. M., J. A. Cunningham, G. Droguett, E. A. Kurtjones, A. Krithivas, J. S. Hong, M. S. Horwitz, R. L. Crowell, and R. W. Finberg.** 1997. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. Science **275:**1320–1323.
4. **Bergelson, J. M., J. G. Mohanty, R. L. Crowell, N. F. St. John, D. M. Lublin, and R. W. Finberg.** 1995. Coxsackievirus B3 adapted to growth in RD cells binds to decay-accelerating factor (CD55). J. Virol. **69:**1903–1906.
5. **Boere, W. A., B. J. Benaissa-Trouw, T. Harmsen, T. Erich, C. A. Kraaijeveld, and H. Snippe.** 1986. The role of complement in monoclonal antibody-mediated protection against virulent Semliki Forest virus. Immunology **58:** 553–559.
6. **Boyer, V., C. Desgranges, M. A. Trabaud, E. Fischer, and M. D. Kazatchkine.** 1991. Complement mediates human immunodeficiency virus type 1 infection of a human T cell line in a CD4- and antibody-independent fashion. J. Exp. Med. **173:**1151–1158.
7. **Boyer, V., C. Delibrias, N. Noraz, E. Fischer, M. D. Kazatchkine, and C. Desgranges.** 1992. Complement receptor type 2 mediates infection of the human CD4-negative Raji B-cell line with opsonized HIV. Scand. J. Immunol. **36:**879–883.
8. **Cardosa, M. J., J. S. Porterfield, and S. Gordon.** 1983. Complement receptor mediates enhanced flavivirus replication in macrophages. J. Exp. Med. **158:** 258–263.
9. **Chow, L. H., K. W. Beisel, and B. M. McManus.** 1992. Enteroviral infection of mice with severe combined immunodeficiency. Evidence for direct viral pathogenesis of myocardial injury. Lab. Invest. **66:**24–31.
10. **Cooper, N. R.** 1991. Complement evasion strategies of microorganisms. Immunol. Today **12:**327–331.
11. **Coyne, K. E., S. E. Hall, S. Thompson, M. A. Arce, T. Kinoshita, T. Fujita, D. J. Anstee, W. Rosse, and D. M. Lublin.** 1992. Mapping of epitopes, glycosylation sites, and complement regulatory domains in human decay accelerating factor. J. Immunol. **149:**2906–2913.
12. **Crowell, R. L., A. K. Field, W. A. Schleif, W. L. Long, R. J. Colonno, J. E. Mapoles, and E. A. Emini.** 1986. Monoclonal antibody that inhibits infection of HeLa and rhabdomyosarcoma cells by selected enteroviruses through receptor blockade. J. Virol. **57:**438–445.
13. **Dempsey, P. W., M. E. D. 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:**348–350.
14. **de Verdugo, U. R., H. C. Selinka, M. Huber, B. Kramer, J. Kellermann, P. H. Hofschneider, and R. Kandolf.** 1995. Characterization of a 100-kilodalton binding protein for the six serotypes of coxsackie B viruses. J. Virol. **69:**6751–6757.
15. **Devine, D. V., K. Wong, K. Serrano, A. Chonn, and P. R. Cullis.** 1984. Liposome-complement interactions in rat serum: implications for liposome survival studies. Biochim. Biophys. Acta **1191:**43–51.
16. **Fearon, D. T., and R. M. Locksley.** 1996. The instructive role of innate immunity in the acquired immune response. Science **272:**50–53.
17. **Grist, N. R., and D. Reid.** 1993. Epidemiology of viral infections of the heart, p. 23–31. *In* J. E. Banatvala (ed.), Viral infections of the heart. Edward Arnold, a division of Hodder and Stoughton, London, England.
18. **Hirsch, R. L., J. A. Winkelstein, and D. E. Griffin.** 1980. The role of complement in viral infections. III. Activation of the classical and alternative complement pathways by Sindbis virus. J. Immunol. **124:**2507–2510.
19. **Klingel, K., S. Stephan, M. Sauter, R. Zell, B. M. McManus, B. Bultmann, and R. Kandolf.** 1996. Pathogenesis of murine enterovirus myocarditis: virus dissemination and immune cell targets. J. Virol. **70:**8888–8895.
20. **Knowlton, K. U., E. S. Jeon, N. Berkley, R. Wessely, and S. Huber.** 1996. A mutation in the puff region of VP2 attenuates the myocarditic phenotype of an infectious cDNA of the Woodruff variant of coxsackievirus B3. J. Virol. **70:**7811–7818.
21. **Kosco, M. H., A. P. Monfalcone, A. K. Szakal, and J. G. Tew.** 1988. Germinal center B cells present antigen obtained in vivo to T cells in vitro and stimulate mixed lymphocyte reactions. Adv. Exp. Med. Biol. **237:**883–888.
22. **Lederman, S., M. J. Yellin, L. R. Covey, A. M. Cleary, R. Callard, and L. Chess.** 1993. Non-antigen signals for B-cell growth and differentiation to antibody secretion. Curr. Opin. Immunol. **5:**439–444.
23. **Lindberg, A. M., R. L. Crowell, R. Zell, R. Kandolf, and U. Pettersson.** 1992. Mapping of the RD phenotype of the Nancy strain of coxsackievirus B3. Virus Res. **24:**187–196.
24. **Liu, Y. J., J. Zhang, P. J. Lane, E. Y. Chan, and I. C. MacLennan.** 1991. Sites of specific B cell activation in primary and secondary responses to T cell-dependent and T cell-independent antigens. Eur. J. Immunol. **21:**2951–2962.
25. **Lodge, P. N., M. Herzum, J. Olszewski, and S. A. Huber.** 1987. Coxsackievirus B-3 myocarditis: acute and chronic forms of the disease caused by different immunopathogenic mechanisms. Am. J. Pathol. **128:**455–463.
26. **MacLennan, I. C., Y. J. Liu, and G. D. Johnson.** 1992. Maturation and dispersal of B-cell clones during T cell-dependent antibody responses. Immunol. Rev. **126:**143–161.
27. **Mapoles, J. E., D. L. Krah, and R. L. Crowell.** 1985. Purification of a HeLa cell receptor protein for group B coxsackieviruses. J. Virol. **55:**560–566.
28. **McChesney, M. B., and M. B. Oldstone.** 1987. Viruses perturb lymphocyte functions: selected principles characterizing virus-induced immunosuppression. Annu. Rev. Immunol. **5:**279–304.
29. **Montefiori, D. C., K. Stewart, J. M. Ahearn, J. Zhou, and J. Zhou.** 1993. Complement-mediated binding of naturally glycosylated and glycosylation-modified human immunodeficiency virus type 1 to human CR2 (CD21). J. Virol. **67:**2699–2706.
30. **Oldstone, M. B.** 1996. Virus-lymphoid cell interactions. Proc. Natl. Acad. Sci. USA **93:**12756–12758.
31. **Pepys, M. B.** 1976. Role of complement in the induction of immunological responses. Transplant. Rev. **32:**93–120.
32. **Prohaszka, Z., T. Hidvegi, E. Ujhelyi, H. Stoiber, M. P. Dierich, C. Susal, and G. Fust.** 1995. Interaction of complement and specific antibodies with the external glycoprotein 120 of HIV-1. Immunology **85:**184–189.
33. **Shafren, D. R., R. C. Bates, M. V. Agrez, R. L. Herd, G. F. Burns, and R. D. Barry.** 1995. Coxsackieviruses B1, B3, and B5 use decay accelerating factor as a receptor for cell attachment. J. Virol. **69:**3873–3877.
34. **Szakal, A. K., K. L. Holmes, and J. G. Tew.** 1983. Transport of immune complexes from the subcapsular sinus to lymph node follicles on the surface of nonphagocytic cells, including cells with dendritic morphology. J. Immunol. **133:**1714–1727.
35. **Szakal, A. K., J. K. Taylor, J. P. Smith, M. H. Kosco, G. F. Burton, and J. J. Tew.** 1990. Kinetics of germinal center development in lymph nodes of young and aging immune mice. Anat. Rec. **227:**475–485.
36. **Szakal, A. K., M. H. Kosco, and J. G. Tew.** 1988. A novel in vivo follicular dendritic cell-dependent iccosome-mediated mechanism for delivery of antigen to antigen-processing cells. J. Immunol. **140:**341–353.
37. **Tew, J. G., M. H. Kosco, and A. K. Szakal.** 1989. The alternative antigen pathway. Immunol. Today **10:**229–232.
38. **Tew, J. G., M. H. Kosco, G. F. Burton, and A. K. Szakal.** 1990. Follicular dendritic cells as accessory cells. Immunol. Rev. **117:**185–211.
39. **Tomko, R. P., R. Xu, and L. Philipson.** 1997. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. Proc. Natl. Acad. Sci. USA **94:**3352–3356.
40. **van den Eertwegh, A. J., J. D. Laman, M. M. Schellekens, W. J. Boersma, and E. Claassen.** 1992. Complement-mediated follicular localization of T-independent type-2 antigens: the role of marginal zone macrophages revisited. Eur. J. Immunol. **22:**719–726.
41. **van Rooijen, N.** 1990. Antigen processing and presentation in vivo: the microenvironment as a crucial factor. Immunol. Today **11:**436–439.
42. **Vuorinen, T., R. Vainionpaa, R. Vanharanta, and T. Hyypia.** 1996. Susceptibility of human bone marrow cells and hematopoietic cell lines to coxsackievirus B3 infection. J. Virol. **70:**9018–9023.
43. **Vuorinen, T., R. Vainionpaa, H. Kettinen, and T. Hyypia.** 1994. Coxsackievirus B3 infection in human leukocytes and lymphoid cell lines. Blood **84:**823–829.
44. **Wolfgram, L. J., K. W. Beisel, A. Herskowitz, and N. R. Rose.** 1986. Variations in the susceptibility to coxsackie virus B3-induced myocarditis among different strains of mice. J. Immunol. **136:**1846–1852.