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Activation of the Immune Coagulation System by Murine Hepatitis Virus Strain 3

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Viral infections result in alterations in hemostasis and coagulation. It has previously been shown that susceptibility to murine hepatitis virus strain 3 (MHV-3), a coronavirus, correlates directly with the spontaneous, T lymphocyte–instructed expression of a procoagulant monokine that exhibits prothrombin-cleaving activity (procoagulant activity [PCA]). A biologic role for PCA in the pathogenesis of MHV-3 infection is suggested by results of in vivo microscopic observations made during acute MHV-3 infection. Recently, it has been demonstrated that prostaglandin E_2 abrogates the induction of PCA by MHV-3 both in vivo and in vitro and prevents hepatic necrosis and the associated microcirculatory changes. These data suggest that MHV-3 induces cellular injury through the activation of the coagulation cascade and provide further evidence for a role for PCA in the pathogenesis of MHV-3 infection.

The Immune Coagulation System

The coagulation cascade is known to be an important component of many inflammatory reactions [1]. These reactions include delayed-type hypersensitivity reactions, the response to infections, and the response to allograft rejection, in which fibrin deposition plays a major role [2–4]. Deposition of fibrin provides the molecular basis for the induration of these lesions, which is considered the requisite phenotypic feature. Several coagulation pathways have been implicated in the pathogenesis of the extravascular deposition of fibrin. Increased capillary permeability observed in inflammatory lesions may expose the contact factors of plasma to activators such as collagen in the extravascular space. Alternatively, the cells of the inflammatory lesion may supply one or more procoagulants capable of activating clotting further down the cascade. White blood cells have been known to influence the local coagulation of blood. Monocytes and macrophages involved in inflammatory responses have been demonstrated to express procoagulants on their cell surface. This procoagulant activity (PCA) can be generated both in vitro and in vivo by mononuclear cell cultures in response to endotoxin, mitogens, antigens, immune complexes, allogeneic cells, infectious agents, and proteolytic products of the complement cascade (figure 1) [5–10].

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Pathways for Induction of Cellular Procoagulants

The cellular pathways responsible for these responses to diverse biologic stimuli have now been examined [11]. Over the past several years, studies have provided insight into the cellular responses necessary for induction of monocyte/macrophage procoagulants; T lymphocyte–monocyte interactions have been implicated, although a T lymphocyte–independent pathway has been described.

Pathway I: The rapid response. First, a very rapid PCA response is elicited by lipopolysaccharides (LPS), immune complexes, and possibly syngeneic tumor cells. A full response is observed within 4–6 hours and culminates in the synthesis of a procoagulant that initiates the coagulation protease cascade. In humans, the procoagulant is tissue factor, whereas in mice the primary effector molecule is a direct prothrombinase. The monocyte/macrophage is the cellular source of the activity, but T lymphocyte collaboration is required for expression. The T lymphocyte responsible can be defined by passage through nylon wool followed by segregation on a fluorescent-activated cell sorter with use of monoclonal antibodies to helper T cells (Leu 3A, OKT4). The instructor T cell also is Fcμ-positive and forms rosettes with erythrocytes. We have now identified the existence of suppressor T lymphocytes for the LPS-driven PCA response, thus providing further

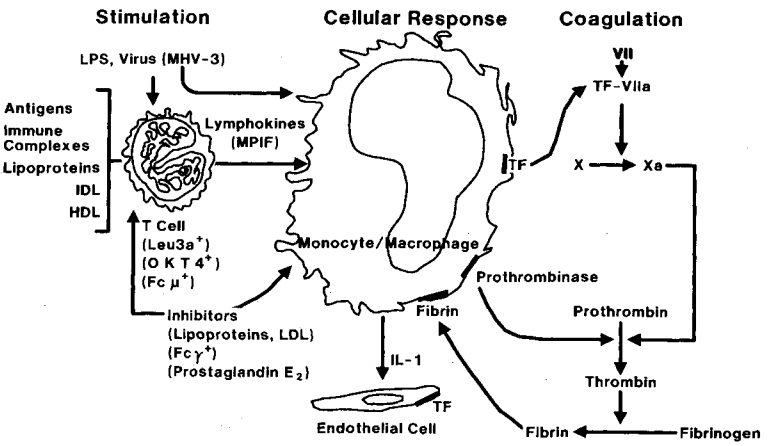

Figure 1. Immune regulation of induction of monocyte/macrophage procoagulant activity. The pathways involved in the induction and regulation of monocyte/macrophage procoagulant activity are represented, along with the site of action of the procoagulants in the coagulation cascade. MPIF = monocyte procoagulant-inducing factor; LPS = lipopolysaccharide; LDL = low-density lipoproteins; IDL = intermediate-density lipoproteins; HDL = high-density lipoproteins; MHV-3 = murine hepatitis virus strain 3; IL-1 = interleukin 1; TF = tissue factor.

evidence for the regulatory aspects of the cellular procoagulant response.

Pathway II: The slow allogeneic response. A recently delineated second pathway of PCA response differs from the first in most details of its operation. Allogeneic stimulation in vivo generates PCA-expressing monocytes, some of which have been shown to selectively localize to the rejecting allograft [12, 13]. The kinetics for induction of this pathway is significantly slower than that for the first pathway, requiring ~72 hours for a response in a human two-way mixed lymphocyte culture. This pathway is highly specific, unlike the first pathway, which requires only the lipid moiety of LPS.

Pathway III: The response to antigens. Recent studies by a number of investigators have demonstrated a procoagulant response to antigen. The existence of such an antigen-driven PCA immune response has been suggested by the observations of Dvorak et al. [14, 15]. This pathway differs from the previously described responses: the temporal kinetics are slower (days), and the cellular interactions that take place are different. Monocytes must first present soluble protein antigens to T lymphocytes in a step that requires cell contact. T lymphocytes that have been stimulated in this way communicate with monocytes to elicit induction of PCA. Proliferation of T lymphocytes is not required, since mitomycin-treated lymphocytes are fully capable of mediating the response.

Pathway IV: Other T lymphocyte-dependent responses. Finally, we have recently described an antigen-specific PCA response to murine hepatitis virus strain 3 (MHV-3) that correlates with the severity of the liver disease induced by the virus in the mouse [9, 16, 17]. Genetic restriction of both the primary and secondary PCA immune responses as well as of the type of monocyte/macrophage product (PCA and plasminogen activator) produced determines the pathogenetic features. The details of these pathways are less well defined and may embody the characteristics of a number of the already defined pathways. Although these pathways appear to operate (at least in part) via lymphokine-producing T cells, direct monocyte/macrophage induction of PCA appears to be a factor.

Pathway V: The lymphocyte-independent PCA response. A number of investigators have suggested that monocytes and macrophages not only synthesize procoagulant activity but are directly induced to synthesize procoagulants without the cooperation of other cells; thus, lymphocytes may be essential in the collaboration but not in the induction of PCA [18–20].

Nature of Procoagulants

On the basis of results from the fibrin-specific immunofluorescence studies of Hogg [21, 22] and the single-cell fibrin plaque assay we have developed [5], it is now clear that generation of PCA leads directly to the formation of fibrin on the surface of monocytes or macrophages. However, the nature of the PCA generated by monocytes/macrophages is less clear. Different procoagulants have been described for cells from different species, different anatomic locations, or stimulated by different agonists under different conditions either in vitro or in vivo. At least three different procoagulants have been described, the nature of which is dependent on the protein fac-

tors of the coagulation system [23]. The first, tissue factor, which has been defined by its dependence on factor VII for expression of PCA, is also characterized by its insensitivity to phospholipase C and by resistance to serine protease inhibitors [24]. Tissue factor is the major procoagulant of human monocytes/macrophages. A second procoagulant induced by LPS in rabbit hepatic macrophages has been characterized by Maier and Ulevitch as a direct activator of factor X [20]. Furthermore, Shands has provided preliminary data characterizing a macrophage-associated procoagulant (also a direct activator of factor X) in mouse peritoneal macrophages stimulated with lipopolysaccharide in vitro [25]. A third procoagulant, a direct prothrombin activator, has been described in human and mouse peripheral blood monocytes grown in tissue culture [26].

Significance of Procoagulant Activity

The biologic significance of the PCA response rests on the demonstration that the cellular PCA response is probably central to the classic delayed-type cutaneous hypersensitivity lesion [11, 27–30]. In addition, when elicited by LPS, this cellular response appears to be a primary pathway underlying the pathogenesis of the classical Shwartzman reaction (disseminated intravascular coagulation or consumption coagulopathy) [31, 32]. A number of inflammatory and immune disorders, including allograft rejection, systemic lupus erythematosus, glomerulonephritis, and viral hepatitis (in both humans and mice) are associated with laboratory evidence of activation of blood coagulation and histologic evidence of fibrin deposition in vivo [33–35].

We have recently demonstrated that the strain-dependent susceptibility of mice to MHV-3 correlates directly with the T lymphocyte-controlled expression of a procoagulant monokine that exhibits prothrombin-cleaving activity after stimulation by MHV-3 both in vitro and in vivo [9, 17]. Increased activity of plasminogen activator has been found in monocytes from resistant mice at a time when PCA was markedly elevated in susceptible animals. A pathogenic role for PCA has been suggested by in vivo microscopic observations during acute MHV-3 infection in fully susceptible BALB/cJ mice and semisusceptible C3H/St mice.

The present article describes the effects of MHV-3 on induction of PCA and on the microcirculation of the liver and the role of prostaglandins in modulating the expression of PCA and in preventing disease during viral infection.

Murine Hepatitis Virus

Coronaviruses constitute an important group of pathogens in both human and animal hosts. Coronaviruses are responsible primarily for respiratory and enteric disease, depending on the strain, host species, and route on inoculation [36–40]. Different strains of coronavirus within a single host species can assume varied tropisms.

MHV-3, a member of the coronavirus family, produces a strain-dependent spectrum of liver disease in inbred strains of mice [16, 41]. Mice of the A strain are fully resistant to the effects of the virus, whereas BALB/cJ mice and C57Bl/6J mice are fully susceptible to the virus and die of fulminant hepatic necrosis. Animals of the C3H strain survive the acute phase of the illness, progress as chronic carriers of the virus, and develop chronic inflammatory disease of the liver. It is now apparent that virus replicates in both hepatocytes and macrophages from all three strains of mice; thus, viral replication and expression of target viral antigens on infected hepatocytes is not in itself sufficient for injury.

Within 72 hours of infection with 10^3 pfu of MHV-3, necrotic lesions are found in the livers of BALB/cJ mice. These lesions become progressively more severe, and by the fifth day after infection, the animals die of fulminant hepatic necrosis. When immunofluorescence studies that use antisera to plaque-purified HMV-3 are performed, large quantities of viral antigens are observed in the livers of all animals by 24 hours after infection. In contrast, no evidence of liver damage is observed in A/J mice, although significant amounts of virus are present (MHV antigens were demonstrated by immunofluorescence). C3H mice develop acute liver disease that is considerably less severe than disease in BALB/cJ mice by day 4 and subsequently progress to the chronic carrier state, with histologic evidence of chronic hepatitis. Relatively severe vasculitis, which is most prominent in the area of the terminal portal venules, is observed in the livers of C3H mice 21 days after infection with MHV-3. This vasculitis is associated with subendothelial deposits of fibrin. Virus can be detected by immunofluorescence in the livers of infected C3H mice for up to 4 months after initial infection.

The Procoagulant Immune Response in MHV-3 Infection

Procoagulant Activity and Susceptibility to MHV-3

We have observed that peripheral blood mononuclear cells isolated from the blood of mice and stimulated in vitro with MHV-3 respond with an increase in PCA. This response correlates directly with susceptibility of animals to hepatic injury [9, 16, 17] (table 1). Peripheral blood mononuclear cells respond in a vigorous fashion with a dose-dependent PCA response that is demonstrable within 1–2 hours after exposure to the virus; maximum levels are attained at 12 hours. The PCA response persists for 48 hours with no diminution in activity. A significant but smaller increase in PCA occurs in peripheral blood mononuclear cells from C3H mice, whereas no response is found in cells isolated from A/J mice, even in the presence of up to 10^7 pfu of MHV-3, a difference correlating with resistance to disease. In contrast, mononuclear cells from all three strains of mice produce a vigorous and equivalent PCA response to LPS. Mononuclear cells from all three strains of mice fail to produce a PCA immune response to ultraviolet-irradiated and noninfectious MHV-3.

To determine whether the PCA response to MHV-3 infection would occur in vivo, we isolated peripheral blood mononuclear cells and splenic cells from mice after their infection with MHV-3 and assayed for both cell-surface PCA and total PCA (table 1). Peripheral blood mononuclear cells from BALB/cJ, A/J, and C3H mice injected with normal saline as controls had a basal surface PCA of 0.8 mU/10^6 peripheral blood mononuclear cells. Cells from BALB/cJ mice that had been infected with 10^3 pfu of MHV-3 exhibited a 120-fold increase in surface PCA and a 200-fold increase in total PCA (relative to basal PCA) at 4 hours postinfection. Cellular PCA continued to rise to a maximum activity of 7,500 mU/10^6 peripheral blood mononuclear cells for surface PCA and 12,500 mU/10^6 peripheral blood mononuclear cells for total PCA—a 120-fold increase at 48 hours postinfection—a level that persisted until death on day 5 postinfection. No increase in PCA was observed in either peripheral blood mononuclear cells or splenic mononuclear cells from the disease-resistant A/J mice for up to 10 days after infection with 10^3 pfu of MHV-3. Peripheral blood mononuclear cells from C3H mice responded with a significant increase in cellular PCA that was less than that seen in BALB/cJ mice. A 10-fold increase in surface PCA and a 40-fold increase in total cellular PCA were observed 12 hours postinfection; this continued to a 20-fold increase in surface PCA and to a 30-fold increase in total PCA by 72 hours postinfection. The elevated PCA persisted at these high levels until 7 days postinfection. On day 10 there was a fall in cellular PCA, but it continued at 10- to 20-fold above basal PCA and correlated with the presence of chronic inflammatory lesions found in both liver and extrahepatic tissues. Episodic increases in PCA that appeared to correlate with a histologically demonstrated increase in inflammatory cells and liver injury were occasionally observed. Similar elevations were detected in splenic mononuclear cells isolated from responding mouse strains, but the PCA generated was less than that seen in peripheral blood mononuclear cells.

When cells were fractionated into lymphocyte and monocyte or macrophage populations, PCA was localized to the monocyte or macrophage populations; there was no increase in the small amount of PCA in the lymphocyte-enriched fractions.

Table 1. Correlation between hepatitis and induction of procoagulant activity (PCA) by murine hepatitis virus strain 3 (MHV-3).

Mouse strain	PCA (mU/10^6 mononuclear cells)				Pathologic findings
			MHV-3		
	Control	LPS*	In vitro†	In vivo‡	
A/J	110	2,850	115	120	Normal
BALB/cJ	125	2,250	8,950	15,000	Fulminant hepatic necrosis
C3H/St	115	2,340	1,850	3,450	Chronic hepatitis

* 1×10^6 peripheral blood mononuclear cells were cultured in the presence of 10 μg of bacterial lipopolysaccharide (LPS) (*Escherichia coli* 0111) for 6 hours and assayed for PCA.

† 1×10^6 cells were stimulated with 10^6 pfu of plaque-purified MHV-3, and PCA was determined.

‡ Mice were infected with 10^3 pfu of plaque-purified MHV-3, and mononuclear cells were isolated and assayed for PCA.

Genetic Linkage of Procoagulant Activity to MHV-3 Infection

The mode of inheritance of susceptibility or resistance to MHV-3 has been determined by typing the set of AXB/BXA recombinant inbred strains derived

from resistant A/J and susceptible C57Bl/6J progenitors for susceptibility to infection, as determined by the severity of the pathologic state of the liver [42]. The strain distribution pattern for susceptibility shows a discontinuous variation: one strain is fully resistant (A-like), four strains are fully susceptible (B-like), and 17 strains demonstrate an intermediate degree of susceptibility (figure 2). The fully susceptible strains develop fulminant hepatitis that results in death; mice of the fully resistant strain do not develop liver disease, whereas mice of the semisusceptible strains develop disease ranging from mild focal hepatitis to widespread hepatocellular necrosis. This strain-dependent pattern best fits the two-recessive-gene model of inheritance, and neither of these two loci is linked to the H-2 complex. Monocyte/macrophage PCA is segregated among the recombinant inbred strains in a strain distribution pattern identical to that of susceptibility and resistance. PCA levels are elevated more than sevenfold in fully susceptible recombinant inbred mice and more than fourfold in semisusceptible mice; there is no increase of PCA levels in resistant mice. These observations suggest genetic linkage of susceptibility and resistance to MHV-3 infection and monocyte/macrophage PCA.

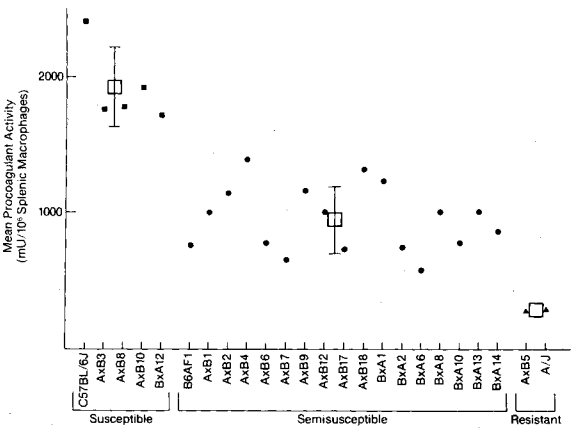

Figure 2. Association of procoagulant activity (PCA) with susceptibility/resistance to infection with murine hepatitis virus strain 3 in the inbred and recombinant inbred (RI) strains of mice. Four RI strains of mice and the parental strain (C57BL/6J) that were fully susceptible to infection with MHV-3 expressed high levels of PCA; 17 RI strains (all of which developed chronic disease) expressed an intermediate PCA response; in contrast, one RI strain and the parental A/J strain that were fully resistant to infection had no increase in PCA.

MHV-3 Infection and the Microcirculation of the Liver

To determine whether the alterations in monocyte or macrophage PCA could have an effect on liver structure and hepatic vascular perfusion during the course of MHV-3 infection, we observed the microcirculation in anesthetized, ventilated animals with use of a fiber-optic quartz rod and video camera time-lapse microscopy; images were stored on video tape [43, 44]. In uninfected A/J, BALB/cJ, and C3H mice, normal streamlined blood flow featuring alternating terminal hepatic venules and terminal portal venules was observed. Sinusoidal flow was readily seen in transit from the terminal portal venules to the terminal hepatic venules and individual hepatocytes were visualized.

Acute Changes

Marked abnormalities in liver blood flow were observed in BALB/cJ mice within 6–12 hours of MHV-3 infection. Initially, granular flow attributed to clumping of erythrocytes was observed in both afferent and efferent blood vessels. This change was followed by focal enlargement of hepatocytes and the appearance of sinusoidal microthrombi. These microthrombi were most evident in the periportal regions. By 24–36 hours after infection, focal areas of enlarged hepatocytes and little or no sinusoidal blood flow were observed. By 48 hours after infection, areas of apparent necrosis were seen where microthrombi had apparently compromised the vascular perfusion of a group of sinusoids (figure 3). At 72 hours, widespread hepatic necrosis was seen. The earliest abnormalities observed in the hepatic microcirculation preceded evidence of viral replication in the hepatocytes or histologic abnormalities.

Chronic Changes

In C3H mice, granular blood flow was observed by 12 hours after infection. Hepatocellular enlargement and sinusoidal microthrombi were present at 24 hours postinfection. Small foci of hepatocellular necrosis were seen at 48 hours, but our studies demonstrated that by day 7 new areas of infection—characterized by granular blood flow, hepatocellular swelling, and microthrombi—were observed in areas other than those of resolving inflammation.

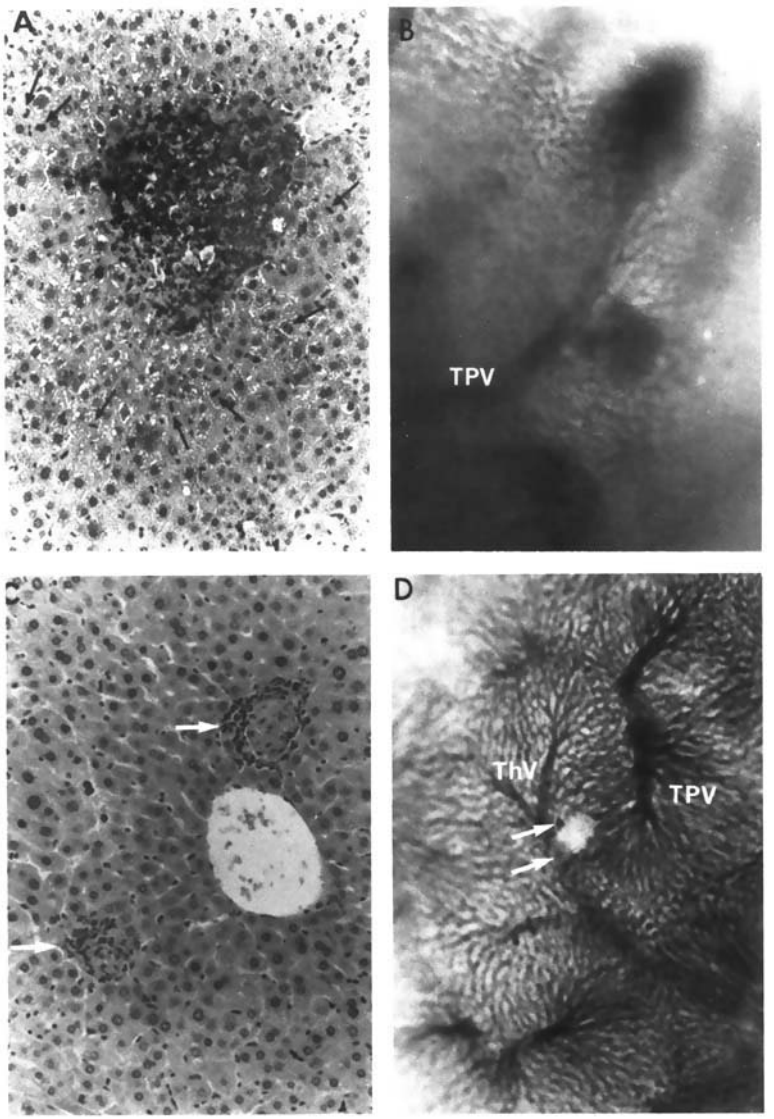

Figure 3. Studies of the livers of mice after infection with murine hepatitis virus strain 3 (MHV-3). *A*: Dense mononuclear cell infiltrate with numerous mitotic figures in hepatocytes at 2 days postinfection in BALB/cJ mice (hematoxylin and eosin, original magnification, ×700). *B*: In vivo microscopy at 2 days postinfection in the same section demonstrating hemorrhagic foci of necrosis adjacent to a terminal portal venule (TPV) (original magnification, ×550). *C*: Epithelioid granulomas scattered throughout the liver parenchyma 30 days postinfection in C3H mice (hematoxylin and eosin; original magnification, ×800). *D*: In vivo microscopy at 30 days postinfection in the same section in C3H mice demonstrating a pale, well-circumscribed avascular lesion with pigmented cells at the margin. Terminal portal venule and terminal hepatic venule (ThV) are as indicated (original magnification, ×350).

This pattern of episodic recurrence of acute disease with chronic inflammation persisted over the next 6–9 months until death of the animal. In areas of chronic inflammation (usually referred to as granulomas), blood flow was granular and hepatocellular swelling and microthrombi were prominent at the periphery of the lesions, with multicell-thick plates in the center of the lesions (figure 3). In A/J mice, despite the presence of virus in the livers (as demonstrated by the recovery and growth of infectious particles and by the presence of viral antigens both in the cytoplasm and on the surface of hepatocytes by immunofluorescence), blood flow remained streamlined throughout the course of the infection and no microthrombi or vascular abnormalities were observed.

This deleterious effect of viral infection on the microcirculatory vasculature is not unique to infection with MHV-3. A number of investigators have noted severe effects on endothelial cell integrity and function after viral infection. These include the findings of viral particles and disturbances of morphology in endothelial cells [45–47]. After parvovirus infection, cushion-like swellings of endothelial cells that produce focal narrowing of the vascular lumen are noted. The vessels become lined by pyknotic, desquamated endothelial cells or by denuded endothelium. During the early stages of vascular ob-

struction, leukocyte aggregates that adhere to the vessel walls can be seen in capillaries and venules. Subsequently, aggregated erythrocytes and fibrin thrombi occlude the parenchymal arterioles [48].

Effect of Prostaglandins on the Course of MHV-3 Infection and on Induction of PCA

Prostaglandins and Viral Infections

Prostaglandin is a generic term for a family of bioactive lipids derived from arachidonic acid via the cyclooxygenase pathway. The biologic activities attributed to these compounds are both varied and heterogeneous. A number of investigators have demonstrated the cytoprotective effects of prostaglandins—particularly 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$)—in gastric mucosa [49], the liver [50], and the kidney [51] in response to a variety of toxins and other noxious stimuli. In addition, prostaglandins have been shown to have varied effects on smooth muscle, particularly with respect to changes in the tone of blood vessel musculature [52]. Furthermore, it is generally accepted that prostaglandins exert a predominantly suppressive effect on the immune system, although the mechanism by which this occurs is poorly understood [53]. It has been demonstrated that many macrophage-activation parameters are modulated by prostaglandin E_2 (PGE_2) [54, 55]. Recently, both endogenous and exogenous arachidonic acid metabolites (prostaglandins of the E series) have been shown to affect the expression of class II histocompatibility molecules by murine macrophages [56]. Reports of the effect of products of arachidonic acid metabolism on viral infection remain controversial. Prostaglandins of the E series have been shown to enhance the spread of herpes simplex virus in cell cultures [57] and have been implicated indirectly as immunosuppressive agents in MHV-3 infection [58]. Other investigators have shown that prostaglandins of the A series are potent inhibitors of Sendai virus replication in vitro [59]. This antiviral action is not specific and is not influenced by the inability of the cell to produce interferon. Furthermore, in vitro treatment with prostaglandins of the E series reduces the replication of

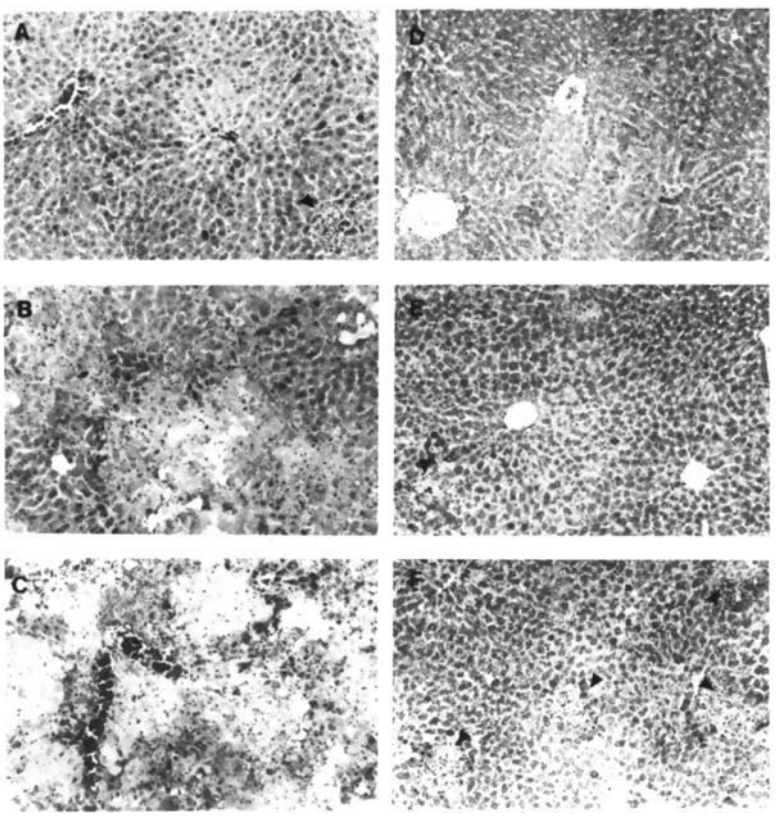

Figure 4. The effect of dimethyl prostaglandin E_2 ($dmPGE_2$) on liver histology after infection with murine hepatitis virus strain 3. Liver sections after infection demonstrated scattered, small foci of inflammatory cells (arrow) at 48 hours (*A*), widespread necrosis at 72 hours (*B*), and confluent necrosis at 96 hours (*C*). In contrast, concomitant treatment with $dmPGE_2$ results in normal histology at 48 hours (*D*), only an occasional focus of inflammation at 72 hours (*E*), and scattered foci at 96 hours (*F*) (hematoxylin and eosin; original magnification, ×600).

Table 2. The effect of prostaglandin E_2 (PGE_2) on infection with mouse hepatitis virus strain 3 (MHV-3).

Treatment group	Serum alanine aminotransferase (IU/L)	Morphometry (% necrosis)	Viral titer (log_{10} pfu/g of liver ± SEM)	Procoagulant activity ($mU/10^6$ macrophages)
PGE_2 alone	20 ± 15	0	0	46 ± 20
MHV-3 alone	1,402 ± 619	93	$2 \times 10^8 \pm 1 \times 10^7$	615 ± 262
MHV-3 + PGE_2	63 ± 40	24	$6 \times 10^8 \pm 1 \times 10^8$	96 ± 70

NOTE. These data represent peak values obtained 4 days postinfection.

mengo, MM, and polio viruses in association with an enhanced yield of interferon and an inhibition of cell division [60].

Effect of Dimethyl Prostaglandin E_2 on MHV-3 Infection

We have now demonstrated, by both biochemical and histologic findings, that $dmPGE_2$ is able to prevent the development of fulminant hepatitis (figure 4) in BALB/cJ mice after infection with MHV-3 without altering viral replication or infectivity [61, 62]. Similarly, splenic mononuclear cells recovered from mice infected with MHV-3 and treated with $dmPGE_2$ demonstrated no augmentation in PCA; in contrast, mononuclear cells recovered from mice infected with MHV-3 and not treated exhibited a marked rise in the level of PCA (table 2). Furthermore, $dmPGE_2$ was able to prevent the induction of PCA in monocytes/macrophages isolated from BALB/cJ mice and stimulated with MHV-3 in vitro. The lack of a detectable protective effect by $dmPGF_{2\alpha}$ in our studies points to the heterogeneous properties of prostaglandins. The cytoprotective effect of $dmPGE_2$ on hepatocyte cultures infected with MHV-3 in vitro suggests that $dmPGE_2$ acts, at least in part, directly at the level of the hepatocyte; in addition, however, our data suggest that $dmPGE_2$ may also influence the immune response to MHV-3.

Conclusions

These studies demonstrate that induction of a PCA response appears to be a marker for disease activity in murine viral hepatitis and may be genetically linked to the genes for resistance and susceptibility. In MHV-3 infection, the induction of PCA antedates both viral replication and histologic abnormalities. After MHV-3 infection, severe microcirculatory abnormalities occur concomitantly with the induction of the PCA response in circulating monocytes but before the appearance of other features of viral infection. Thus, it appears that the capacity of monocytes to initiate the coagulation-protease cascade could be significant in the pathogenesis of MHV-3 infection by (*1*) leading to local protease generation, (*2*) augmenting other cytotoxic mechanisms, (*3*) indirectly inducing platelet aggregation and the release of bioactive products, and (*4*) initiating local microthrombus formation, which interferes with effective perfusion and nutrition of individual cells.

The observation that viral infection results in direct destruction of endothelial cells [48] is consistent with the findings of our studies of the effect of MHV-3 on the microcirculation and lends further support for the role of the immune-coagulation system in the pathogenesis of viral disease. In addition, the ability of the monocyte/macrophage to initiate the coagulation protease cascade in the vascular stream, to localize and initiate the local deposition of fibrin, or even to participate directly in cellular injury by protease augmentation of cytolytic activity may represent a central pathogenetic mechanism. Furthermore, the expression of procoagulant monokines with resultant alterations in vascular permeability and integrity may be inhibited by mediators such as prostaglandins or leukotrienes. These mediating agents would conceivably prevent cell injury and death.

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