VIRUSES AND THE VERSATILE MACROPHAGE

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Mononuclear phagocytes, including circulating monocytes and tissue macrophages, play a central role in resistance to viruses. This resistance can be expressed both non-specifically, and specifically in induction, regulation and amplification of humoral and cell mediated immune responses to viruses. These lead to the extrinsic effect of macrophages on other virus-infected cells or free virus, and the intrinsic effect on viruses within macrophages. While these interactions usually appear to be protective, immunopathologic consequences as well as macrophage dysfunctions have also been noted. The outcome of any given interaction (viral elimination, persistence, latency or transformation) varies markedly with the type of macrophage. The molecular mechanisms involved in these very diverse macrophage-virus interactions are currently under study.

Mononuclear phagocytes (MP) comprise a widely distributed cell system that includes immature cells in the bone marrow, circulating monocytes and tissue macrophages (Mφ). Mφ are placed strategically throughout the body to meet foreign particles, and are prominent in the liver, lung, spleen and body cavities. The importance of MP in non-specific resistance to viruses was emphasized first in the 1960s.¹⁻³ Now it is recognized that they also play a central role in the induction, regulation and amplification of specific immune responses, as well as being pivotal in nonspecific resistance, homeostasis and synthesis of potent biologic mediators. The importance of MP perhaps can be appreciated best by their very early phylogenetic appearance, and the fact that no genetic defect resulting in absence of MP has yet been found in vertebrates that is compatible with life.

Current Concepts of the Role of MP in Resistance to Viruses

The reader is referred to several recent reviews of the role of MP in resistance to herpes simplex virus (HSV) and other viruses for most work published prior to 1982.⁴⁻⁶ Extensive data document the importance of MP in resistance to virus infections in animal models, and probably in human disease (Table 1).⁴⁻⁸ It has been difficult to deplete MP selectively in order to determine their precise role, but a new experimental system may prove to be useful—treatment of mice with radioactive ⁸⁹Sr to destroy bone marrow and thus remove marrow dependent cells. We have found that ⁸⁹Sr causes a rapid and profound decrease in circulating monocytes, polymorphonuclear leukocytes and in NK cell activity in CD1 mice, but does not have significant effects on the number or

function of tissue Mφ.⁹ Moreover, natural or immunomodulator-enhanced resistance to encephalomyocarditis (EMC) virus¹⁰ and HSV (unpublished observations) was not markedly changed from that in non-treated mice. These data provide evidence for a prominent role for tissue Mφ in non-specific resistance to these viruses.

It is becoming increasingly apparent that MP do not act in isolation. There is a dynamic and balanced interplay of MP with other resistance mechanisms, most notably natural killer cells and specific cell mediated and humoral immune responses (see pp. 22-27 & 92-97).^{6,11-14} Delineating the relative roles for the various immune elements *in vivo* is a major research goal. *In vitro* systems have provided experimental approaches to define these elements in isolation and in controlled interactions.

Intrinsic and Extrinsic MP-Virus Interactions In Vitro

Extrinsic resistance of MP is defined as their ability to inactivate extracellular virus or reduce production in other surrounding cells that are normally permissive. The *intrinsic* interaction is defined as the permissiveness/non-permissiveness of the MP itself for growth of a virus. The extrinsic ability of MP to suppress virus production in another cell is independent of the ability of the MP to support virus replication itself.^{6,15} The interactions may be completely non-specific, or may be modified immunologically in either direction.

The extrinsic interaction may be modified by specifically immune cells, antibody, complement or lymphokines. T lymphocytes may activate MP for increased antiviral activity, thus amplifying specific T cell immunity.¹⁶ The activity of low levels of specific antibody can be amplified by MP-mediated antibody dependent cellular cytotoxicity (ADCC) for virus infected cells.^{14,17} When primary cultures of MP are used, it can be difficult to isolate the effects of MP alone from combined effects with these other immunologic elements or contaminating lymphoid cells.¹⁸⁻¹⁹ Unfortunately, there have been few studies of the extrinsic interaction of pure cultures of bone-marrow-derived Mφ (BMDMφ), Mφ clones or Mφ-like cell lines with virus infected cells. Such studies will be invaluable in defining at the molecular level the mechanisms involved in MP mediated extrinsic antiviral activity.

Viruses that have been shown to be inhibited by extrinsic MP effects include HSV, cytomegalovirus (CMV), Marek's disease, mouse hepatitis (MHV), ectromelia, EMC and vesicular stomatitis viruses (VSV).^{4,6,15,20-22} Several operative mechanisms have been demonstrated (Table 2). Undoubtedly multiple processes are involved in limiting such diverse virus groups, e.g., if a virus has a high requirement for arginine, then arginine depletion by arginase secreted by MP may be important.²³ The mechanism in any given interaction appears to differ depending upon the particular MP, and the particular 'Achilles heel' of the virus replication strategy in the permissive cell. It is not yet clear whether diverse functional

Table 1

Evidence for involvement of mononuclear cells in resistance to viruses in experimental animal models

Monocytes and Mφ predominate at sites of viral infection
Mφ transfer resistance, particularly in neonatal animals which form a model of Mφ deficiency
Mφ often required for T cell or antibody transfer of resistance
Mφ activated during virus infection for increased antiviral activity
Mφ activation by immunomodulators, although other cells (e.g. NK cells) may also be activated, increases resistance
Mφ depletion may decrease resistance
Increased Mφ antiviral action (e.g. age, genetic factors) often correlates with increased resistance

Table 2

Reported mechanisms of extrinsic mononuclear phagocyte-mediated antiviral activity

Direct inactivation of extracellular virus by enzymes, reactive oxygen metabolites or other factors secreted by Mφ
Inhibition of virus adsorption and penetration into permissive cells
Lysis of virus-infected cells prior to production of new virus
Enzymatic depletion of molecules needed for virus replication, e.g. arginine
Synthesis of interferon that inhibits virus replication in the permissive cells
Inhibition of synthesis of macromolecules necessary for virus replication in the permissive cells
Inhibition of virus maturation in the permissive cells
Inhibition of virus release from the permissive cells

mechanisms co-exist within the same MP cell, or if these functions are mutually exclusive, since antiviral activity is not necessarily correlated with other MP functions.^{4,6,22}

In MP-virus intrinsic interactions there are also a multitude of possible outcomes. These include abortive, cytolytic, persistent non-cytolytic, and latent infections, and transformation. The non-permissiveness of MP for most viruses has been the subject of numerous investigations.^{4,6,24,25,113} MP-mediated oxidative metabolism, which is important in some tumoricidal and microbicidal activities, was not important for intrinsic resistance to VSV infection in parental or oxidative metabolism-defective cloned Mφ-like cell lines.²¹ A possible role for MP secretion of arginase has also been studied, but conflicting results have been obtained.^{23,26,27}

Interferons produced by MP appear to play a complex role in intrinsic resistance. Interferon had no effect on African swine fever virus,²⁸ but decreased production of rubella virus in MP.²⁹ Interferon induced by a high multiplicity of infection with HSV was correlated with inhibition of productive virus replication in human monocytes.³⁰ The authors suggested that interferon caused antiviral activity and also inhibition of monocyte to Mφ differentiation in vitro. Whether the endogenous interferon production was related to defective interfering particles remains to be determined. The use of anti-interferon serum treatment in vivo has suggested that endogenous interferon may be present under some physiologic conditions and maintain resident peritoneal Mφ (ResMφ) in a nonpermissive state for certain viruses.^{31,118} The recent finding that human MP infected with different viruses produce different alpha interferons with distinctive antiviral spectra is also of interest.³² The interaction of alpha/beta interferon, but not gamma interferon, with the Mx gene for resistance to influenza virus in Mφ, is an elegant example of the complex mechanisms governing the outcome of intrinsic Mφ-virus interactions.^{33,119}

MP Heterogeneity in Intrinsic Interactions with Viruses

MP are morphologically and functionally very diverse and the origins of this diversity have not yet been delineated.³⁴ Recent studies have established the effect of in-vitro 'ageing' and the maturation of monocytes to Mφ in the Mφ-virus interaction. Ageing in culture appears to increase replication of HSV,²⁶⁻³⁰ caprine arthritis-encephalitis virus,³⁵ and rubella virus²⁹ in MP. What relationship ageing in vitro has to in-vivo differentiation/maturation of MP is unresolved.¹²⁰ Cells susceptible to infection and transformation by avian myeloblastosis virus (AMV) have been found at all stages of MP differentiation.³⁶

Regardless of the stage of differentiation only a subset of Mφ are targets for transformation by AMV.³⁷ The same has been described for productive infection with HSV,⁶ CMV,^{38,39} lactic dehydrogenase (LDH),^{40,41} rubella²⁹ and influenza viruses.⁴² Most laboratories have reported that only 3-20% of the MP are

infected. With LDH, the Mφ permissiveness for virus has been both reported to be related to,¹¹⁶ and not to be related to Ia antigen expression (Brinton, personal communication). The reasons for the apparent permissiveness and non-permissiveness within a Mφ population remain an enigma. It is not known whether the heterogeneity is related to cell cycle, to stages of differentiation, to separate sublines of Mφ, or transient modulation by environmental factors.

In addition to the heterogeneity that exists among MP naturally in vivo or through culture in vitro, MP can be altered by a myriad of treatments in vivo and in vitro. Immunization increases intrinsic resistance of Mφ to some viruses,^{6,43} but not others.^{24,55} Intrinsic resistance of Mφ to HSV was reported to be decreased in rats bearing a transplantable epithelioma.⁴⁴ Treatment of the MP in vitro with cytochalasin B or phytohaemagglutinin has been reported to alter permissiveness.^{29,45} The use of agents to elicit tissue Mφ, however, is probably a major source of variability in virus-Mφ interactions observed among different laboratories, and this must be recognized when comparing various systems of virus-Mφ interactions.⁶

An interferon-mediated mechanism in Mφ for the immunomodulator, *Corynebacterium parvum* (CP), has been reported recently for ectromelia virus,²⁰ but does not appear to be effective for MHV. In general the attempts to use agents such as CP, interferon, or lipopolysaccharide,²⁴ to elucidate the mechanisms for Mφ intrinsic resistance, have provided more confusion than consensus.

There have been few studies of possible changes in the intrinsic resistance of non-dividing MP when the cells are put under the proliferative stimulus of colony-stimulating factor. Proliferating BMDMφ appear to be more susceptible to guinea pig herpes-like virus,⁴⁸ and MHV.⁴⁹ Proliferation of variously elicited peritoneal Mφ resulted in increased production of LDH and Sindbis viruses,⁴⁷ but not of HSV.⁵⁰ Results with LDH suggest generation of new transiently permissive cells from non-permissive precursors (116 and Brinton, personal communication). We have found major differences between ResMφ and BMDMφ in their interactions with HSV (see later section). Whether elicitors and MP growth stimulators increase the mitotic index of tissue Mφ, which is then responsible for increased viral infection, is an intriguing possibility. Efficiency of infection and transformation with AMV was directly related to the mitotic activity of MP.³⁶

The above highlights have pointed out the diversity in MP from the same body site, and how these cells are altered by various treatments. There is also heterogeneity in permissiveness of MP from different organs. For example, mouse alveolar and peritoneal Mφ are reported to respond very differently to infection with influenza virus,⁴² while LDH appeared to replicate equally well in Mφ from different organs.⁴¹

The genetic background of the animal provides a final source of variation in the virus-Mφ response. In a few instances, e.g. MHV⁵¹ and Rift Valley fever viruses (Rosebroeck and Peters, personal communication), the resistance of the animal to infection paralleled MP resistance to the virus. More commonly, the genetic background affects all cells in a relatively equal fashion.⁵²

In summary, MP constitute a very diverse population of cells. Compounding this natural heterogeneity with the imposed heterogeneity of experimental systems precludes drawing broad conclusions regarding mechanisms of Mφ-virus interactions (Table 3). Where data are most intriguing, investigators have endeavoured to isolate and thoroughly characterize a single animal-virus system.³⁶ Likewise, where in-vivo infections are mimicked by in-vitro results, there is reassurance of the validity of the in-vitro observations.^{40,42} New tools now being developed will aid in

Table 3

Experimental factors that affect mononuclear phagocyte-virus interactions

Virus Factors
Strains
Passage history
Mutation
Multiplicity of infection
Defective interfering particles
MP Factors
Organ source of MP peritoneal, blood, bone marrow, lung, spleen, liver
Animal species
Animal age neonatal, adult
Genetic background
Culture conditions in vitro medium, duration, adherent or suspension culture
Treatment of animals in vivo elicitation with thioglycollate broth, mineral oil, proteose peptone, activation with <i>C. parvum</i> , BCG, immunization with live or killed viruses
Treatment of Mφ in vitro lipopolysaccharide, interferon, macrophage activating factor
Mφ proliferation
Mφ subpopulations size, Ia antigens, ectoenzymes, Mφ differentiation antigens

defining the interactions of Mφ and viruses. Monoclonal antibodies to MP have the potential to separate and identify subpopulations of MP heterogeneous in functional phenotypes, as well as tissue-types, and elicited and resident Mφ.³⁴ The studies of the effects of local microenvironment on expression of various markers and ectoenzyme profiles hold great promise^{114,115} in determining the origins of functional variability of the MP branch of the immune network.

Pathologic Effects of MP in Virus Infections

As with lymphocyte responses, MP responses can either be beneficial or harmful. T lymphocyte-activated MP can eliminate virus and be useful in most extraneural sites, but are pathologic in the central nervous system (see pp. 75-79). Similarly, while MP-mediated ADCC lyses virus infected cells and thus decreases virus, ADCC can also cause massive tissue necrosis.

In acute infection of adult mice with CMV, activated Mφ can clearly be protective.^{4,6} At the same time, it has been documented that splenic Mφ are a major site for productive CMV infection and, if mice are splenectomized, resistance to CMV is increased.⁵³ Other work has shown that only a small percent of the Mφ are cytolytically infected,⁵⁴ and that Mφ may be the predominant site for latent infection with CMV and guinea pig herpes-like virus.^{48,54,55} Thus, CMV may replicate preferentially in a subpopulation of splenic Mφ and BMDMφ, while other Mφ are relatively resistant. The role of latent viral infection in MP as a means of harbouring potentially activatable virus merits study with other viruses in addition to these two herpesviruses.

Another type of pathologic interaction is exemplified by visna-maedi retrovirus infection in sheep and goats.⁵⁶ The virus produces a persistent non-cytolytic infection in monocytes and tissue Mφ. Thus protected from immune responses, infectious virus is continually disseminated to cells permissive for cytolytic infection. A few other viruses also appear to have a predilection for persistent and often non-cytolytic infection in MP, a mechanism that may be related to infection chronicity (Table 4). In addition, the pathogenesis of scrapie has recently been suggested to involve replication of the agent in MP.⁵⁷

Considerable investigation has been conducted to define antibody enhancement of virus infection of MP. This phenomenon has been reported with a wide range of RNA viruses.⁵⁸⁻⁶³ Enhanced infection requires monocytes, peritoneal Mφ or Mφ-like cell lines with Fc receptors, and non-cytophilic IgG antibody that exhibits virus serotype or cross-reactive specificity.⁵⁸⁻⁶⁵ In one case, some enhancement was also observed with IgM and the CR3 complement receptor.⁶⁶ The effective virus multiplicity of infection is apparently increased. Increasing phagocytosis by

Table 4

Viruses reported with predilection for persistence or latent infection in mononuclear phagocytes

Virus	Virus nucleic acid	Group	Reference
Lactate dehydrogenase virus	RNA	Togavirus	40, 41
Mouse hepatitis virus	RNA	Coronavirus	49, 110
Feline infection peritonitis virus	RNA	Coronavirus	95
Sendai virus	RNA	Paramyxovirus	74
Lymphocytic choriomeningitis virus	RNA	Arenavirus	8, 117
Haemorrhagic fever viruses (Lassa fever, Junin, Machupo, Pichinde, Argentinian haemorrhagic fever)	RNA	Arenavirus	82, 111
Avian myeloblastosis virus	RNA	Retrovirus	36, 37
Equine infectious anaemia virus	RNA	Retrovirus	4
Friend leukaemia virus	RNA	Retrovirus	112
Visna-maedi	RNA	Retrovirus	56
Cytomegalovirus	DNA	Herpesvirus	39, 54
Aleutian mink disease virus	DNA	Parvovirus	4
African swine fever virus	DNA	Indovirus	28

treatment of Mφ with activating agents also increased virus uptake and replication.⁶⁷ It is hypothesized that dengue virus haemorrhagic fever, in individuals undergoing a second infection, involves non-neutralizing antibody which enhances virus infection in monocytes. The infected monocytes then become targets for T cell immune elimination and release inflammatory mediators.⁶⁸⁻⁶⁹ MP can be extremely sensitive targets for histocompatibility-restricted T cell cytotoxicity, presumably because of the prominent expression of major histocompatibility complex antigens on the MP surface.¹¹ There is an obvious need to determine whether this phenomenon plays a general role in viral pathogenesis.

Virus Effects on MP Functions

This section briefly updates Mogensen's recent review.⁷⁰ Virus infected cells can produce chemotactic stimuli or inhibitors.^{4,70-72} Moreover, the intrinsic interaction of viruses with MP may alter MP behaviour (Table 5). Cytolytic infection of MP removes the MP system in a local or systemic manner. The subsequent decreased resistance can be demonstrated by frog virus 3 destruction of hepatic Kupffer Mφ, allowing hepatocytes to become available for vaccinia virus infection.⁷³ The more subtle outcomes of intrinsic interactions, i.e. abortive or persistent infections, can also alter MP functions (Table 4). Depressed function of alveolar Mφ after infection of mice with CMV, Sendai, or influenza viruses has been associated with predisposition to secondary microbial infection in the lung.^{70,74} Studies in vitro have focused on changes in MP receptors, phagocytosis, oxidative metabolism, phagosome-lysosome fusion and microbicidal killing.^{70,75-80} Usually depressed function has also been observed, although no effect and even enhanced activity has been noted. These conflicting results may be related to different Mφ populations, to Mφ differentiation, or to the particular virus used.

Other MP activities may be affected by virus infection. Mice acutely or chronically infected with MHV or Sendai virus showed impaired wound healing, which could be overcome by local administration of Mφ stimulating agents.⁸¹ Infection of Mφ with Pichinde virus,⁸² Newcastle disease or lymphocyte choriomeningitis viruses⁷⁰ inhibited Mφ proliferation in response to colony stimulating factor. We have also observed that MHV infection changes the ectoenzyme phenotype of ResMφ to that of activated Mφ, and may inhibit proliferation of BMDMφ (Dempsey, unpublished observations).

Few studies have addressed virus effects on MP immunoregulation, which may play a central role in viral pathogenesis and in immunopathologic responses. Monocytes or Mφ infected with CMV, influenza, Sendai or poliovirus have been shown to be suppressive

Table 5

Functions of mononuclear phagocytes that may be affected by virus infection

Chemotaxis
Attachment and phagocytosis of particles through nonspecific, Fc or complement receptors
Intracellular oxidative response
Lysosome-phagosome fusion
Intracellular microbicidal activity
Synthesis and/or secretion of biologically active molecules, e.g. prostaglandins, neutral proteases, interferon and complement
Antigen presentation
Regulation of immune responses, i.e. accessory and suppressor activity
Mφ activation process for microbicidal and tumoricidal activity
Antibody dependent cytotoxicity (ADCC) by Mφ
Wound healing
DNA synthesis and Mφ proliferation in response to macrophage growth factor
Mφ differentiation

for certain lymphocyte responses.⁸³⁻⁸⁵ LDH infection has been reported to interfere with MP antigen presentation.⁴¹ Bovine rhinotracheitis and HSV infections have been shown to inhibit Mφ ADCC activity.⁷⁶⁻⁷⁸ A recent report indicates that only early CMV protein synthesis, without any apparent morphologic change in the Mφ, was required for Mφ dysfunction.^{86a} The relevance that such Mφ immunoregulatory changes may have during viral infections requires further investigation.

Use of Molecular Biology Techniques to Define Heterogeneity

The preceding sections have reviewed the extensive data available on the biological interactions of Mφ and viruses. There is now a distinct need to detail the precise mechanisms at the molecular level. The heterogeneity of Mφ is well known, but the gene expression involved in the evolution of this heterogeneity is poorly understood. The overall rate of RNA synthesis in polyinosinic-polycytidylic acid activated Mφ, elicited with proteose peptone, is decreased.^{86b} Concomitantly, there is an increased rate of glucose oxidation and cytolytic activity and perhaps an increased rate of protein synthesis. This suggests a selective turn-on/off of specific gene products during Mφ activation. Recent work, using recombinant cDNA clones of mRNAs in activated RAW 264.7 Mφ-like cells, has identified a family of coordinately regulated mRNAs. Two dimensional gel analysis of proteins in these cells shows the same coordinate regulation of protein synthesis (Largen, personal communication). These are important beginnings in the definition of the molecular mechanisms of Mφ heterogeneity and differentiation.

Use of Molecular Biology Techniques to Define Mφ-virus Interactions

The elucidation of Mφ-virus interactions is approaching adolescence. We are beginning to note the terms 'viral genomes', 'use of cloned probes', 'synthesis of mRNA transcripts' etc. For example, Haller et al. discovered that Mφ bearing the Mx loci, upon stimulation with interferon, could no longer replicate influenza virus.⁵² Virus penetration was normal, yet no viral proteins were synthesized.⁸⁷ When the cells were analyzed by RNA/RNA hybridization, normal influenza virus transcripts were present, which could not be translated. In a cell-free in-vitro translation system, virus-specific proteins were synthesized from these mRNAs.³³ A unique protein induced by interferon in Mx-bearing cells has been identified,⁸⁸ what selective action it may have on the translation of primary influenza virus transcripts remains undetermined.

Selected restriction of viral replication by different populations of Mφ has also been studied by molecular biology techniques.

Table 6

Interaction of HSV-1 with various mononuclear phagocytes and Vero cells

Cells		Hours after infection		
		2	24	48
Vero	CPE*	—	++	++++
	HSV genome†	10-100	10,000	10,000
ResMφ	CPE	—	—	—
	HSV genome	10-100	10-100	Undetectable
BMDMφ	CPE	—	++	+++
	HSV genome	10-100	100-1000	1000-10,000

* CPE—cytopathic effect where — = 0-10%, + = 10-25%, ++ = 25-50%, +++ = 50-75%, ++++ = 75-100%

† Estimated number of total HSV genomes per diploid cell equivalent. Cells were infected at a multiplicity of infection of 5

MCMV-infected TGMφ and ResMφ showed virus-specific DNA present in approximately 85% of each cell population, but more infectious centres in TGMφ.⁵⁵ Moreover, when TGMφ or ResMφ from latently infected mice were examined, virus was produced only from the TGMφ even though there were low levels of MCMV DNA in both cell types. The authors concluded that activation of Mφ plays a major role in the in-vivo activation of MCMV infection following latency. In another non-Mφ cell line, HCMV also does not replicate unless the cells have been stimulated to differentiate.⁸⁹

Stevens and Cook have shown the presence of HSV DNA and empty virus particles in Mφ unable to replicate HSV.⁹⁰ To examine the mechanism of restriction in more detail, we compared the replication of HSV-1 in BMDMφ, ResMφ and permissive Vero cells (Table 6). At 24 hour post infection (PI) in Vero cells, the cells showed considerable cytopathic effect (CPE) and a yield of HSV of about 10⁷ PFU/culture (100 PFU/cell). The BMDMφ also showed marked CPE at 48 h PI but no evidence of production of infectious virus, the yield being 10⁴ PFU/culture (0.03 PFU/cell). ResMφ showed no CPE and no evidence of infectious virus (10² PFU/culture, 0.0005 PFU/cell). Using cloned Eco RI fragments of the HSV-1 genome⁹¹ as radioactive probes in cell and dot-blot analysis, 60% of the HSV-1 genome (Eco RI fragments D, G, N, F, M, O, A and I) was present in all cell types at 2 h PI. In BMDMφ, viral DNA replication occurred; the number of viral genome equivalents per cell increased with time. In contrast, the viral DNA content in ResMφ decreased to undetectable levels by 48 h PI. Eco RI fragments H, L, EK, and JK representing the terminal repeats, joint region and part of unique long region are reported to contain sequences that cross-hybridize with cellular sequences (Sandri-Goldin personal communication).^{92,93} The examination of this region of the HSV-1 genome in infected Mφ by the more detailed Southern blot analysis⁹⁴ showed results similar to those for the other parts of the HSV genome. These data provide evidence that the block in virus production in BMDMφ occurs at a point beyond viral DNA replication, while the block in ResMφ is prior to HSV DNA synthesis. However, we do not know if a subpopulation of BMDMφ replicate HSV-1 DNA.

Use of Mφ Clones and Cell Lines

While a definition of Mφ heterogeneity in molecular terms will enable us to better understand Mφ differences and viral interactions, the development of Mφ lines is essential, particularly for in-vitro studies.

Most investigations in intrinsic interactions have used non-dividing monocytes or tissue Mφ, which are relatively non-permissive for most viruses, such as VSV and HSV. The available data with Mφ-like cell lines indicate that permissiveness for VSV and HSV is not solely dependent upon the ability of Mφ to divide.

J774.16 cells showed relative non-permissiveness for VSV,²¹ similar to ResMφ.³¹ There is variable permissiveness for HSV among Mφ-like cell lines.^{6,50} WEHI-3 were as non-permissive as ResMφ, while mouse PU5-1.8 and human U937 cells were moderately permissive, although not as much as Vero cells.⁶ As with non-dividing Mφ, usually only a small percentage of any of these cells are productively infected. The metabolic changes related to cell cycle that may be involved in permissiveness/non-permissiveness in cloned cell lines merits additional study.

Several viruses, particularly MHV and LDH, appear to infect Mφ preferentially in vivo, but their replication in Mφ-like cell lines is variable. Feline infectious peritonitis virus replicated well in the few feline Mφ-like cell line.⁹⁵ LDH has not been demonstrated to replicate in a large group of Mφ-like cell lines (Brinton, personal communication). The replication of MHV in Mφ-like cell lines has not been investigated in detail.

Many of the Mφ-like cell lines currently available have evolved as a result of transformation by viruses. Whether MP are more or less permissive for viral transformation than other cells is not known. Simian virus40 (SV40) transforms many cell types including Mφ.⁹⁶ The efficiency of transformation can be greatly increased by transfection with SV40 DNA containing a defective DNA replication origin (SV40 ori⁻).⁹⁷ Two murine Mφ cell lines from BMDMφ or splenic Mφ and one human monocyte/Mφ cell line have been developed by transfection with SV40 ori⁻ DNA.^{98,99} When a non-Mφ cell line was transformed in this manner, its capacity to differentiate was not altered even though it actively expressed SV40 T antigen.¹⁰⁰ Whether all MP properties are also retained in such transformed cells should be carefully monitored. Nonetheless, in addition to the currently available cell lines, it should now be possible to generate a variety of new Mφ-cell lines at various stages of differentiation whose ability to permit virus replication can be studied in detail. Regulation of the control of virus replication can then be examined by inducing each of these cells to differentiate in vitro beyond their 'frozen' state.

In addition to transformation of cells by SV40, the retroviruses have contributed to the generation of immortalized Mφ-like cells.¹⁰¹ However, there is now strong evidence to suggest that the cellular oncogenes (related to the transforming genes of the retroviruses) play a major role in the control of differentiation of Mφ and other cells.¹⁰² Some evidence for this can be derived from temperature sensitive mutant studies on myeloproliferative sarcoma virus transformation of fibroblasts and haematopoietic cells.

The same gene function(s) appear to be involved in the transformation of the two very different cell types.¹⁰³ In the haematopoietic precursor cell line 416B, the levels of endogenous cellular P21 *sar*c (immunologically related to the *src* gene product of Harvey murine sarcoma virus) may be related to differentiation.¹⁰⁴ In the promyelocytic cell line, HL60, regulation of cellular oncogenes is seen following induction of differentiation with dimethyl sulphoxide or retinoic acid.^{105,106} While regulation of differentiation by cellular oncogenes is not restricted to the MP lineage, it clearly plays an important role. With this knowledge, it may now be possible to predict and test for some of the steps involved in MP differentiation, following the examples set for non-MP cells.

Other methods for the generation of Mφ cell lines include the formation of somatic cell hybrids^{107,108} or clones.¹⁰⁹ LDH has been reported to replicate transiently in a mouse Mφ-human fibroblast hybrid.¹⁰⁸ The recent work of Johnson et al. has successfully demonstrated that normal Mφ may be cloned and propagated continuously without the complications of viral transformation and somatic cell hybridization.¹⁰⁹

Concluding Remarks

Before the versatile MP system can be manipulated successfully for immunotherapy against virus infections, methods need to be established to increase MP resistance and decrease immunopathologic outcomes. We have reviewed much data concerning the biological interactions of viruses or virus-infected cells with MP, and the importance of the differentiated and/or activated state of that MP to the outcome of the interaction. A crucial issue now is delineation of the origins of diversity of these cells at the molecular level. Only then will the control of MP gene expression, which determines the differing degrees of extrinsic and intrinsic MP antiviral activity, become evident. The field is now primed for this type of detailed experimental analysis that should lead to potent immunotherapeutic manipulation of the MP system.

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