THE INFLAMMATORY AND IMMUNE RESPONSE TO MOUSEPOX (INFECTIOUS ECTROMELIA) VIRUS

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Summary. – The ectromelia virus (EV) has been recognized as the etiological agent of a relatively common infection in laboratory mouse colonies around the world, i.e., Europe (including Poland), USA and Asia. Due to widespread use of mice in biomedical research, it is important to study the biology of strains characteristic for a given country. This is particularly significant for the diagnosis, prevention and control ectromelia. In severe epizootics, approximately 90% morbidity is observed within colonies and mortality rate exceeding 70% is observed within 4 to 20 days from the appearance of clinical symptoms. The resistance to lethal infection is mouse strain-dependent. Several inbred strains of mice, including C57BL/6 and AKR are resistant to the lethal effects od EV infection, while others, such as A and BALB/c are susceptible. Recent studies indicate that (1) T lymphocytes, NK cells and interferon (IFN)-dependent host defenses must operate for the expression of resistance, (2) virus-specific T-cell precursors appear earlier in regional lymph nodes of resistant than susceptible mice, and (3) resistance mechanisms are expressed during early stages of infection. Over the past several years, (1) induction of anti-EV cytotoxic $CD8^+$ T lymhocytes (CTL) responses *in vivo* in the absence of $CD4^+$ (T helper) cells, (2) importance of some cytokines e.g., IFN-gamma in EV clearance at all stages of infection, and (3) induction of nitric oxide (NO) synthase, which is necessary for a substantial antiviral activity of IFN-gamma, have been demonstrated. The effector mechanisms by which EV-specific immune cells (T lymphocytes) execute their protective and inflammatory functions are thought to involve the release of soluble mediators that attract, focus and activate cells at the infected sites. It is possible that the skin is the most relevant organ for studying the biology of an EV infection *in vivo*, yet very little is known concerning EV replication there and the importance of the skin's innate and immune response for recovery from viral infection.

Key words: ectromelia virus; mousepox; inflammation; immune response

Introduction

"End of the line for smallpox virus?" An editorial article published in 1993 by the "Nature" (Anonymous, 1993) raises the above question as a consequence of the eradication of smallpox in 1967 what led to conclusion in 1986 (World Health Organization Meeting) that the existence of two stocks of the smallpox virus (at the Institute for Viral Preparation in Moscow, Russia and at the Center for Diseases Control in Atlanta, GA, USA) should be physically destroyed, because the nucleotide sequence of the smallpox virus is completely known, and the details incorporated in the appropriate data banks. However, a possible infection with smallpox virus or mutants of the existing poxviruses (i.e. monkeypox virus) is the reason that much may be learned from studies with other animal poxviruses, e.g., mousepox (ectromelia virus, EV).

Instead of smallpox, EV is an excellent candidate for a model virus which should give insights not only into the pathogenesis of mousepox but also help to understand human infectious diseases, namely (1) Molluscum contagiosum which is caused by a poxvirus and is a frequent infec-

tion in the human immunodeficiency virus (HIV)-infected AIDS patients, (2) herpetic (herpes simplex virus type 1 [HSV-1]) skin lesions, and last but not least, (3) smallpox.

Moreover, the mouse is a natural host for EV and is the most popular animal frequently used as experimental model in many areas of scientific research (e.g., biological, medical, veterinary) and industries including pharmaceutical. This is not only due to low cost and relative facile breeding, but also the availability of highly specialized reagents allowing a wide range of investigations and variety of strains and breeds of mice produced by genetic manipulations (e.g., transgenic mice). Mice are even more attractive as experimental models considering their use in drug testing and investigations concerning immune responses that play an important role in formulating strategy for development of vaccines against many infectious agents, e.g., HSV-1 or HIV. Therefore the health of mice before and during investigations is vital for experimental reliability of these animals as well as accurate interpretation of results. In this context, it is wise to be aware of infection with certain viruses that act as a Trojan horse: they can be latent and can be reactivated.

Diseases of mice are caused by many infectious and noninfectious agents, including viruses that can be latent or lead to symptomatic disease. Among the most frequent viruses that infect mice are adenoviruses, pneumonia virus, LDH (lactic dehydrogenase) agent, cytomegalovirus, LIDIM (lethal intestinal disease of infant mice) virus, hepatitis virus, LCMV (lymphocytic choriomeningitis virus), MAIDS (murine retrovirus induced immunodeficiency syndrome) virus, and EV (Jacoby and Fox, 1984; Yetter *et al.*, 1988).

Not only does ectromelia infection undermine the experimental credibility of mice, it also adds to considerable economic loss involving liquidation of colonies and renovation in order to free the housing of the virus. Up-to-date studies show that mousepox is found in many countries, including Poland (Spohr de Faundez *et al.*, 1990). The first incidences of mousepox were reported by Marchal (1930) and Fenner (1949). The Department of Microbiology at the Faculty of Veterinary Medicine of the Warsaw Agricultural University has a longstanding tradition in research on mammalian and avian poxviruses, especially the isolation and biology of Polish strains of EV (for references see Spohr de Faundez *et al.*, 1990).

EPIZOOTIOLOGY, CLINICAL SIGNS, PATHOGENESIS, LABORATORY DIAGNOSTIC AND SCREENING OF MICE

Epizootiology

EV belongs to the family *Poxviridae* and genus *Orthopoxvirus* (Esposito, 1990) (Tables 1 and 2), and is enzootic in certain laboratory mouse colonies. Virus transmission from one colony to another takes place by direct contact of diseased animals or carriers with healthy animals or attendants clothing. The portal of entry are skin abrasions on footpads. After 10 days, characteristic skin lesions appear and infected mice can transmit the virus to healthy animals whithin the first three weeks, which is about the time infected mice pass the virus in their excretion and inflammatory areas on the skin. Also mice that survive the acute phase of the disease may become carriers of EV and a source of infection for healthy animals (Buller *et al.*, 1987; Spohr de Faundez *et al.*, 1990).

Among the known strains of EV, the Moscow strain is the most highly pathogenic (Bhatt and Jacoby, 1987; Bhatt *et al.*, 1988). Other strains such as NIH-79 and strains WAU-86, WAU-88/1, and WAU-88/2/64 isolated in our laboratory are also characterized by high pathogenicity (Spohr de Faundez *et al.*, 1990). Breeds of mice most sensitive to infection are Swiss and inbred strains such as A and A/J (both H-2^a), BALB/c and DBA (both H-2^d), and C3H (H-2^k). The AKR (H-2^k) strains exhibits mild sensitivity, while C57 BL/6 and C57 BL/10 (both H-2^b) are resistant to infection, which is said to be genetically determined (Buller *et al.*, 1986; O'Neill, 1991). It has been shown that (1) T lympfocytes, NK cells and IFN take part in the confrontation with viral infection, and precursors of virus-specific cytotoxic T lymphocytes (CTL-p) appear earlier in regional lymph nodes in resistant than sensitive strains (Buller *et al.*, 1987; Buller *et al.*, 1985; Jacoby *et al.*, 1988; Karupiah *et al.*, 1993*a,b*; O'Neill and Brenan, 1987), (2)

Table 1. Classification and nomenclature of the genus Orthopoxvirus[a]

Family	Subfamily	Genus	Species
Poxviridae[a] or Poxvirus group[c]	*Chordopoxvirinae*[b] or Poxviruses of vertebrates[c]	*Orthopoxvirus*[b] or Vaccinia subgroup[c]	Vaccinia (smallpox vaccine), cowpox (bovines, felines, humans), monkeypox, ectromelia (mousepox), racoonpox, tatera-pox, variola (humans), volepox, vaccinia subspecies (buffalopox and rabbitpox)

[a]Adapted from Esposito, J.J. (1990).
[b]International name.
[c]English vernacular name.

Table 2. Poxviridae – general properties[a]

A. Virus particle:	
1. Morphology	Large, brick-shaped or ovoid virion, 220-450 x 140-260 nm, with external coat containing lipid and tubular or globular protein structures enclosing one or two lateral bodies and a core which contains the genome
2. Physicochemical properties	Ether-resistant or ether-sensitive
3. Nucleic acid	dsDNA, 130 – 375 kbp G+C = 35 – 64% (vertebrate poxviruses) G+C = 20% (entomopoxviruses)
4. Protein	About 4% by weight (vaccinia)
5. Carbohydrate	About 3% by weight
B. DNA replication	In cytoplasm (viroplasm); independent of the host nucleus
C. Cytopathic changes	Cell rounding, granulation and clumping in a number of cell types. Formation of cytoplasmic vacuoles
D. Host range	Vertebrates or invertebrates
E. Transmission	Airborne, contact, fomites, mechanical (i.e., arthropods)
F. Portal of entry into the host	Skin, respiratory tract, oral route

[a]Partially adapted from Buller, R.M.L., and Palumbo, G.J. (1991), and Esposito, J.J. (1990).

defence mechanisms come into effect much earlier in the initial phase of infection in resistant than sensitive mice. Experiments conducted by O'Neill *et al.* (1991) on C57 BL/10 mice indicate that the haplotype H-2 affects the resistance to EV infection but the effects is marginal. Brownstein *et al.* (1991) have pointed out two cardinal genes responsible for infection resistance to be localized in chromosome 2 and 17.

Clinical signs

Mousepox is characterized by at least one of the following symptoms: skin lesions in form of vesicles filled with a serous substance and yellowish crusts on the back skin, tail base, tail, ear lobes, paws, swelling of paws and snout, and conjunctivitis with lacrimation (Spohr de Faundez *et al.*, 1990) (Fig. 1). Noteworthy is the fact that similar lesions may be caused by mouse papulla virus (MPV) which does not have any antigenic relationship with EV. The attention should be paid also to skin damage resulting from biting and amputation of limbs caused by *Streptobacillus moniliformis*, all which might seem to be ectromelia symptoms (Jacoby and Fox, 1984). The mortality in EV infection fluctuates between 1 and 100%. Cells including erythrocytes taken from diseased mice and inoculated into SPF or GF mice may lead to clinical signs characteristic of ectromelia.

Pathogenesis

The incubation period is 7 – 10 days. EV initially replicates in the skin and regional lymph nodes and later appears in the blood stream (primary viraemia). When the virus reaches the liver (Fig. 2) and spleen it replicates intensively in antigen-presenting cells (macrophages and/or dendritic cells) (Spohr de Faundez *et al.*, 1994) (Fig. 3). The process might lead to death of animals due to irreversible damage of these organs. Reappearance of the virus in the blood (secondary viraemia) precedes penetration of the virus into the so-called manifestation organs, especially the skin. Primary lesions on the skin at sites of infection occur 4 – 7 days p.i. and are character-

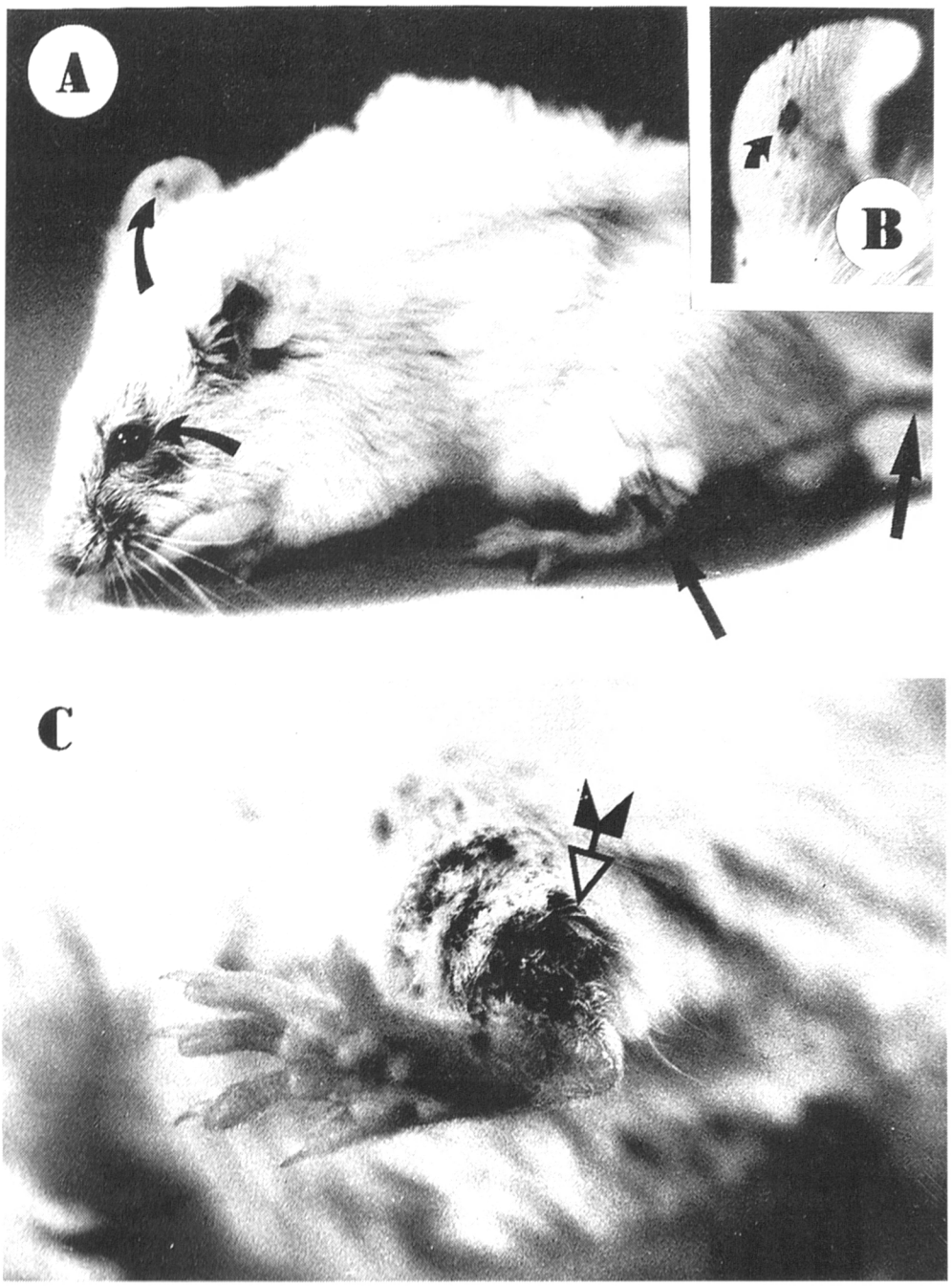

Fig. 1
The BALB/c mouse 14 days after EV foodpad infection
General view (A), skin lesions on the external ear (B), and leg (C). Moscow strain of EV was kindly received from Dr. R.M.L. Buller, NIAID, NIH, Bethesda, MD, USA. Own experiments.

ized by large number of eosinophiles. Skin lesions heal within 2 – 3 days leaving scars. In the acute form of ectromelia, not only necrosis of the spleen and liver is observed, but also lymph nodes, thymus and Peyers patches. Amputation of limbs and tail might also occur.

Laboratory diagnostic

Adoption of precise diagnostic criteria is indispensable, mainly due to possibilities of occurence of non-symptomatic infections which are critical in the light of experimen-

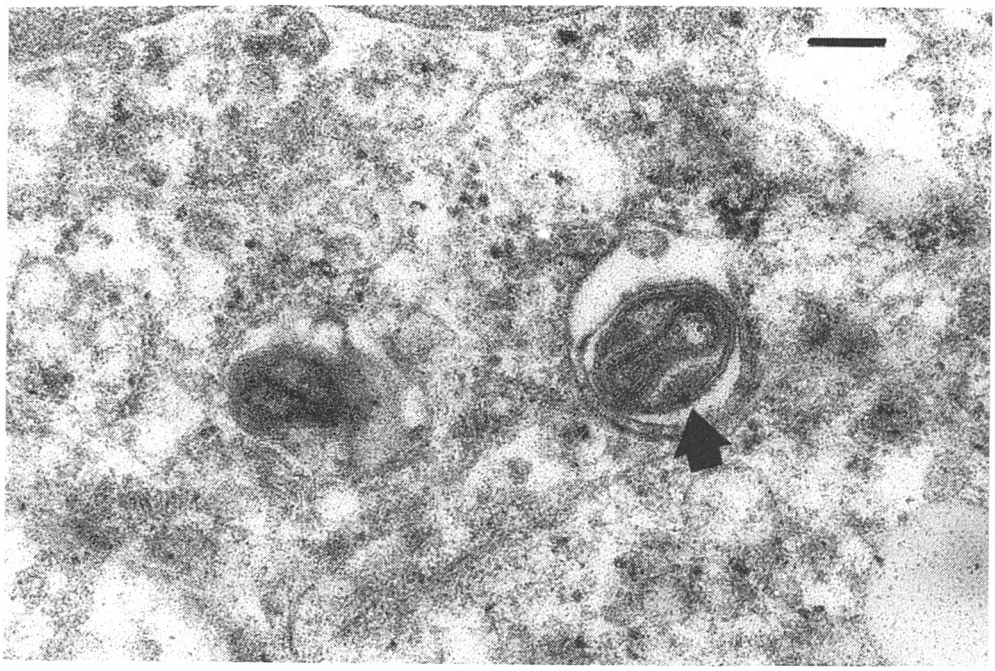

Fig. 2
A transmission electron photomicrograph of Moscow strain of EV in the liver of the BALB/c mouse experimentally infected via footpad 14 days ago
The liver was fixed for 2 hrs in 2.5% glutaraldehyde prepared in sodium cacodylate buffer pH 7.4, postfixed in 1% osmium tetraoxide in the same buffer, dehydrated in graded ethanol solutions and in propylene oxide, and finally embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate. Electron microscope JEOL 100 TEM (Japan) operated at 80 kV. Bar = 100 nm. Own experiments.

tal credibility of the animals. Chen *et al.* (1992) reported that Moscow strain of EV replicates poorly or not at all in cell lines derived from the rabbit or hamster. The failure of EV to replicated in the hamster cells suggested that the virus lacked functional CHO host range (hr) gene required for multiplication in these cells. The diagnosis of ectromelia relies on isolation of virus and it's identification (e.g., by immunofluorescence, transmission electron microscopy, identification of characteristic foci on the chorio-allantoic membrane [CAM] of embryonated chicken eggs) or detection of anti-EV antibodies by seroneutralization [SN], haemagglutination inhibition [HAI] or ELISA (Buller *et al.*, 1983; Czerny *et al.*, 1989; Spohr de Faundez *et al.*, 1990). The diagnostic value of these methods is variable. HAI is less sensitive than ELISA but on the contrary more specific.

Similarly, as in other viral diseases, there is no doubt that the polymerase chain reaction (PCR) will be used in the diagnosis of ectromelia. The available literature does not report the use of PCR pertaining to EV diagnostics.

Screening of mice intended for experiments

Mice originating from outside must be quarantined for at least 7 – 10 days before onset of experiments. This in particular concerns animals obtained from sources other than commercial. In case of disease identification the colony must be eliminated followed by eradication of the virus from infected housing and equipment.

It has been shown that caesarean section does not protect infant mice against infection, because the foetus is usually infected via the intrauterine route. Vaccination with live vaccinia virus (strain IHD-T) adapted to CAM of embryonated chicken eggs may effectively protect mouse colonies in which offsprings had been immunized before 6 weeks of age (Briody, 1959). However, this vaccination does not protect mice against infection. Among conductive factors affecting the appearance of disease and the worsening state of sick animals are physical (X-ray radiation), chemical and biological (e.g., experimental bacterial infections). Castration of animals and stress caused by transportation may initiate the disease leading to an enzootic or epizootic process. Certain bacterial toxins that reduce phagocytic activity of neutrophils and macrophages may increase the sensitivity of mice to EV infection. Care should be taken towards possible transmission of certain poxviruses from animals to humans. This in particular concerns hobbyists or people who profesionally deal with diseased or carrier animals.

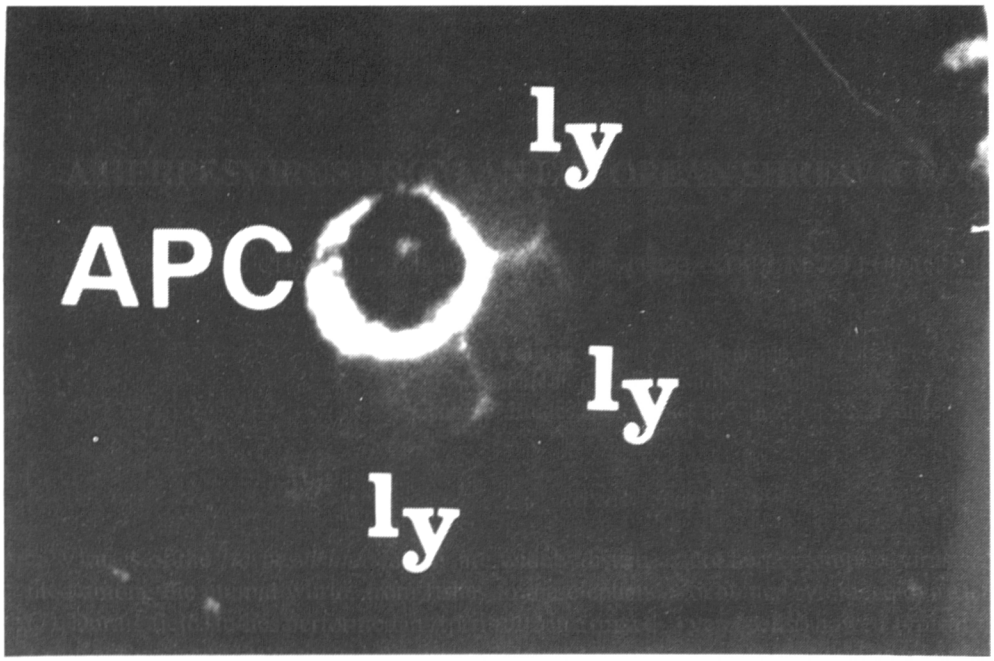

Fig. 3
The rosette phenomenon visible by immunofluorescence
Photomicrograph of antigen presenting cell (APC) and T lymphocytes (ly) clustering during EV-antigen presentation (Vanox Research Photomicrografics Microscope System AHBT3 /Olympus Microscopes, Warsaw/ - kindly accessible by Mr. K. Szukalski). Intimate contacts are made between APC and the surface membranes of the three surrounding T cells. The BALB/c mouse was infected with Moscow strain of EV and 15 days p.i. splenic cells were isolated through 14.5% metrizamide (Nycomed, Pharma AS, Norway) gradient and stained with anti-EV fluorescence conjugate. Original magnification = 600 x. Own experiments.

CELLULAR AND HUMORAL IMMUNITY DURING MOUSEPOX

Cellular immunity

The significant role in immune response during ectromelia, just in other viral diseases, is played by virus-specific CTL (Blanden *et al.*, 1977; Buller *et al.*, 1987; Jacoby *et al.*, 1988; O'Neill and Brenan, 1987). They recognize and destroy infected cells possessing on their surface antigen bound to products of the major histocompatibility complex (MHC). $CD8^+$ CTL recognize viral antigens in the context of class I MHC and $CD4^+$ T lymphocytes through class II MHC. There is a growing recognition of the importance of the regulation of MHC antigen expression by viruses. In contrast to corona- and paramyxoviruses, poxviruses (EV and vaccinia virus) decrease the expression of class I MHC antigen (Maudsley and Pound, 1991). Loss of MHC antigen expression from infected cells may be a strategy for survival or escape from the host immune system.

Three publications by Blanden (1970; 1971*a,b*) concentrated on explanation of cellular defence mechanisms involved in the clearane of EV from organs of mice. In these studies the author relates the use of anti-thymocyte antiserum *in vivo* to eliminate T lymphocytes. Lack of T cells increased sensitivity of mice to EV infection and enhanced virus replication in the liver and spleen thousand times (Blanden, 1970). These results indicate a significant protective role of T cells. Direct substantion of the importance of T lymphocytes during mousepox was associated with transfer of virus-specific T lymphocytes isolated from spleen of diseased mice donors to infected recipient mice in adoptive transfer experiments (Blanden, 1971*a*; Gieryńska *et al.*, 1994). Blanden (1971*a*) confirmed the protective role of virus-specific T lymphocytes that reduced the titer of EV in the liver and spleen of infected recipient mice. The third part of this triptych were histological studies on recipient mice infected with EV, where the author demonstrated the presence of mononuclear cells originating from donor mice (Blanden, 1971*b*). These cells eliminated EV antigens from tissue samples, which was confirmed by negative immunofluorescence.

In 1987, two further publications appeared about cellular immunity in the course of mousepox. The first one presented induction of cytotoxic response ($CD8^+$ CTL) *in vivo* in the absence of helper T lymphocytes $CD4^+$ eliminated by immunosurgery with monoclonal anti-$CD4^+$ antibodies (GK

1.5) (Buller *et al.*, 1987). Authors found that mice ($CD4^+$) were capable of eliciting cytotoxic response against EV which in turn led to recovery, while infected nude mice ($CD4^-8^-$) died. The publication by O'Neill and Brenan (1987) underlined the importance of CTL in EV infection. These authors found that virus-specific CTL-p were present in lymph nodes of mice as early as 2 – 3 days after inoculation with the Moscow strain of EV.

Within the last year, Karupiah *et al.* (1993*a,b*) described (1) the ability of IFN-gamma to inhibit replication of mousepox, vaccinia and HSV-1 viruses in mouse macrophages correlated with production of nitric oxide (NO), and (2) the importance of IFN-alpha, IFN-beta and IFN-gamma in recovery from EV footpad infection of the ectromelia-resistant C57 BL/6 mice. This study underlines the crucial significance of IFN-gamma in EV clearance at all stages of infection.

The effector mechanisms by which EV-specific immune cells (i.e., T lymphocytes) execute their protective and inflammatory functions are thought to involve the release of soluble mediators that attract, focus and activate other cells at the infected sites. Previous studies (Karupiah *et al.*, 1983*b*) have highlighted individual roles of IFN-gamma in this process during EV infection. However, even when EV has been cleared from internal organs (spleen, liver, ovary), high levels of virus infectivity in the skin of the footpad suggest that this primary site of EV replication is protected from antiviral host mechanism including the antiviral CTLs. The means by which this protection is effected is unknown, but it may be mediated, at least in part, by virus-encoded host response modifiers (hrm) – secrected binding proteins for complement (Karupiah *et al.*, 1993*b*), interleukin (IL)-1-beta (Spriggs *et al.*, 1992), tumor necrosis factor (Upton *et al.*, 1991) and IFN-gamma (Upton *et al.*, 1992), blockers of the antiviral action of IFN-alpha/beta (Beattie *et al.*, 1991) and a Crm A gene which inhibits the IL-1 converting enzyme and the generation of dihydroxyeicosatetraenoic acid (diHETE) molecules from the lipoxygenase pathway of arachidonic acid metabolites and inhibitors of arachidonic acid metabolism specifically block orthopoxvirus replication (Palumbo and Buller, 1991; Smith, 1993). It is possible that the skin is the most relevant organ for studying the biology of EV infection, yet very little is known concerning EV replication there and the importance of the skin's innate and immune responses for recovery from viral infection.

Cited publications basically exhaust the available information about cellular defense mechanisms induced by EV antigens. Obviously, these mechanisms are well known in relation to other orthopoxviruses (vaccinia virus) (Binder and Kundig, 1991; Demkowicz and Ennis, 1993; Erickson and Walker, 1993; Spriggs *et al.*, 1992) and certainly some general rules may be applied to immunological reactions taking place during EV infection.

Humoral immunity

Antibodies, as well as the virus itself may be detected by ELISA (Buller *et al.*, 1983; Czerny and Mahnel, 1990; Czerny *et al.*, 1989) or immunofluorescence test (Spohr de Faundez, 1990). The latter is regarded as a sensitive method and worthy recommending for detection of EV antibodies, especially in animals without clinical signs. This method is also considered more reliable than the haemagglutination inhibition (HAI) test. At times the level of antibodies may be very low so that they are undetectable by HAI, however they can be detected by immunofluorescence. ELISA was used to detect anti-EV antibodies by Buller *et al.* (1983), who used the ectromelia or vaccinia viruses purified in sucrose gradient as antigens. It was found that ELISA (titer 1/1000) is at least twenty times more sensitive than the immunofluorescence test (titer 1/40). These studies also showed that anti-EV antibodies can be differentiated from anti-vaccinia antibodies. Recent publications by Czerny and Mahnel (1990), and Czerny *et al.* (1989) show that not only is ELISA cheap, but is also 10 times more sensitive than electron microscopy (EM). To detect orthopoxvirus antigens, EM require the amount of virus in the sample to be at least $10^{-5} – 10^{-6}$/g or ml. Authors obtained monoclonal antibodies against EV, vaccinia and monkeypox.

CONCLUSIONS

The genus *Orthopoxvirus* is a group of related viruses that are serologically difficult to differentiate. These viruses stimulate specific cellular and humoral immune responses, and nonspecific defense mechanisms associated with macrophages, dendritic and NK cells, and also dependent on IFN and other cytokines. Active cellular and humoral immunity can be induced only by live virus, other nonspecific defense mechanisms may as well be stimulated by inactivated virus. Studies with EV should give insights not only into the pathogenesis of mousepox but also help in our understanding of human infectious diseases. Also an exciting development in the genetic manipulation and analysis of immune response of mice with targeted disruptions of genes (i.e., cytokine) opens the possibilities in a whole range of different disciplines in modern biology, also to the viral immunologist working with the animal models.

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