The Enteroviruses: Recent Advances

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New information accrues periodically in the ledger accounting for infections and diseases associated with the human enteroviruses. The discoveries of "new" serotypes and how they affect people are subjects of continuing attention. Some other relevant information on "old" serotypes relates to variations in age-specific attack rates and associated morbidity and mortality for neonates and older infants. Among the morbidity reports are recounts during outbreaks of virus-positive cerebrospinal fluids (CSFs) that initially may not have cytological or biochemical abnormalities. Prolonged enterovirus infections may develop in persons having agammaglobulinemia. Lastly, some provocative associations concern the pathologic expressions of enteroviruses in the development and persistence of injury to the heart (myocardio-pathies) and the pancreas (insulin-dependent diabetes mellitus).

NEW ACQUAINTANCES

The earliest classification of the enteroviruses as polio-, coxsackie, and/or echoviruses has been retained. However, overlapping properties provide difficulties in assigning new serotypes to any one of the sub-groups. Hence new serotypes beginning with Fermon (Enterovirus type 68) have been assigned sequential numbers.

Enterovirus 70

This serotype, responsible for widespread pandemics [1] and constricted focal outbreaks of hemorrhagic conjunctivitis (AHC), was established following recovery of virus from conjunctival scrapings. The disease, characteristically expressed as an ocular infection, occurs in densely populated communities, has high intrafamilial spread, and involves adults more than children. The epidemiologic data indicate eye-hand-vehicular (towels, etc.) modes of transmission.

Appearing first in central Africa, the disease advanced to southeast Asia, and Japan. Despite people migrating from epidemic areas, outbreaks of AHC remained unidentified in the Western Hemisphere until 1981 when outbreaks of AHC occurred in Latin America, in Florida, and in North Carolina [2].

Wadia et al. [3] reported 19 adults (16 males) developing lumbosacral radiculomyelitis two to three weeks after onset of AHC. Motor paralysis (quadramonoplegias) developed in 29 adults during an outbreak in Bangkok. The association was based largely on the development of specific antibodies. Enterovirus 70 was seldom if ever recovered from cerebrospinal fluids; occasionally E-70 was detected in fecal specimens.

Monkeys inoculated intraspinally and intrathalamically with E-70 developed le-
sions in the central nervous system (CNS) similar to those observed for attenuated polioviruses [4]. The disease in monkeys and the neurological findings in human beings suggest that E-70 has neurotropic properties. Whether or not delayed onset radiculomyelitis arises as a consequence of primary viral invasion or from immunological injury remains uncertain.

Sera obtained from domestic animals (cattle, sheep, goats, swine, etc.) in endemic areas neutralize E-70 (titer values > 1:16). One interpretation of the data is that E-70 represents a variant of a parent virus distributed widely in those animals.

**Enterovirus 71**

Schmidt et al. [5] recovered serologically related enteroviruses from 20 persons (1969–1972). These viruses are unrelated to existing serotypes; they have been designated E-71. Sixteen of the 20 isolates (all except one from feces) were derived from children less than 11 years of age. Affected persons had clinical evidence of meningitis or encephalitis. A five-year-old boy died; E-71 was recovered from brain tissue. Deibel et al. [6] recovered E-71 virus from patients having similar illnesses.

Since 1972, outbreaks of disease from E-71 have been reported from Sweden, Japan, Australia, and Bulgaria [7,8]. The clinical features include meningitis, segmental limb paralysis, bulbo-spinal dysfunction, and cutaneous lesions of the hand-foot-mouth syndrome. The epidemic in Bulgaria (1974) mainly involved infants. Most presented with the aseptic meningitis syndrome. Of 451 patients, 100 developed flaccid paralysis or cranial nerve (N VII) palsy or bulbar dysfunction. Twenty-nine of 61 patients (47.5 percent) with brain stem involvement died. Viruses recovered from CNS tissues of 27 persons were identified as E-71.

Enterovirus 71 has a few notable biological properties. The onset and progress of cell destruction in cultured monkey kidney cells varies; cytopathic alterations are less pronounced in rhesus cells than in those derived from grivet or green African monkeys. The Bulgarian strain evokes in monkeys a disease resembling poliomyelitis both clinically and pathologically. Lesions in suckling mice, cotton rats, and hamsters (myositis, paralysis) resemble those of Group A coxsackieviruses.

**ATTENDANT RISKS FOR INFANTS**

Fatal infections from Group B coxsackieviruses and echoviruses types 3, 6, 9, 11, 14, 17, 19, and 31 occur in newborn infants [9]. Exceptional risks relate to concurrent infections in their mothers, to nosocomial events, and possibly to the infants' inability to mount a proper defense response. Otherwise older infants and toddlers may develop overt disease from echoviruses types 3, 6, 11, 16, 18, and 19, as well as enterovirus 71. As a rule illness engendered by many of these viruses has been benign (but see above for E-71); a substantial fraction is associated with fever, rashes, diarrhea, and respiratory illnesses [10,11,12]. Discrete signs of meningitis, even with CNS involvement (i.e., detection of virus) may not be found [12]. The majority of infections are subclinical. Specifically, total recognition is restricted, based on sick infants brought to the clinic or those enrolled in family studies. In our study, specific infections were identified primarily in tests done to rule out sepsis or purulent meningitis. By the midpoint of the outbreak there was a larger clinical appreciation of the prevailing "viral syndrome."

Has there been a shift in attack rates from school age children to susceptible infants and toddlers? The answer to the query is not yet evident, except possibly for some enteroviruses. A major obstacle is the paucity of data relating to immunologic
conversions occurring during infancy and early childhood. Solid information for some enteroviruses (e.g., E-9, etc.) reveals ready recruitment among family associates at risk, thereby resembling polioviruses. Others (e.g., B-3) appear to recruit less extensively and with slower velocities of spread [13,14].

The studies cited above highlight that fraction of the population for which specific illnesses apply. They fail to account for the low-key clinical episodes or silent infections ongoing particularly during outbreaks of enterovirus disease. The relationships between covert and overt infections are well-recognized; however, the true ratios of disease and infection are restricted largely by lack of information on intra-epidemic antibody conversion rates occurring during the period of infancy and early childhood. The real rates are further obscured when combined as they often are in five-year-age spans.

ENTEROVIRUSES RECOVERED FROM “NORMAL” CEREBROSPINAL FLUIDS (CSFs)

The recovery of enteroviruses present in “normal” CSFs is no longer an exceptional event. The first reports involved one or several persons from whom viruses (echoviruses types 4, 6, and 9) were recovered in CSFs unaccompanied by pleocytosis [15]. Later Wilfert et al. [16] recovered echovirus type 18 from 12 of 57 (21 percent) normal CSFs. Bacon and Sims [17] recovered echovirus type 19 from six of 16 (37 percent) such fluids. In our study [12], 22 of 41 (54 percent) normal CSFs were culture-positive for either echoviruses (types 6 and 9) or Group B coxsackieviruses (types 2 and 3). Many, but not all, of the virus-positive CSFs were obtained from infants; six (27 percent) were found among children three to 15 years of age. Although all six were febrile, only two presented with signs of meningitis.

The detection of enteroviruses in “normal” CSFs obtained from sick infants and children again points up obstacles to the correct clinical diagnoses. Signs of meningitis may not be significant components of illness. The CSFs were obtained mainly to exclude early onset bacterial meningitis. The large fractions of positive CSFs cited above provide examples not only for erroneous incidence rates of meningitis, but also of wrong age-specific attack rates reported for infants and young children.

ENTEROVIRUSES AND PERSISTENT INFECTIONS

Immunocompromised persons may react to enterovirus infections differently from their normal peers. The available observations link these lingering infections with gammaglobulinopathies; however, there may be recruitment of persons having combined immunodeficiencies. Described first for the polioviruses, particularly post-vaccination, there are now recounts involving specific enteroviruses; among these are echoviruses type 9, 19, 24, 30, and 33 [18,19,20].

The onset of CNS signs of illness, particularly for polioviruses, where inoculation time is known, may be prolonged (> 3 weeks), with an expanded risk of death and atypical CNS inflammatory lesions. Visceral organs may be involved in disseminated infections; a dermatomyositis-like syndrome may occasionally intervene.

Persons developing poliomyelitis may have prolonged shedding of virus from gut and pharynx; cerebrospinal fluids are virus-negative. In contrast, echoviruses may linger in CSFs for months or years; they are not readily recovered from feces or pharynx. In disseminated forms of infection (e.g., type 24) virus may be recovered from many extraneural organs, including muscle. Dual virus infections have been reported [20].
The peculiar host-parasite relationships are not clearly understandable, since all immunodeficient persons do not seem to have equal risks! An intact B-cell function is undoubtedly essential for blocking access of virus into the CNS, possibly facilitating eradication once therein. The absence of immunoglobulins appears to be a responsible factor in the development of the atypical inflammatory response. Immunosuppressed individuals developing persistent infections may have high risks associated with histocompatibility alleles [20].

ENTEROVIRUSES AND MYOCARDIOPATHIES

Enteroviruses cause not only acute myocardiopathies, but also lingering injury and chronic heart disease [21]. Lerner et al. [22] set criteria associating enterovirus infections with myocardiopathies, ranging from high to low orders of risk. High risks were noted for Group A, types 4 and 16, for Group B, types 1–5 coxsackieviruses, and for echoviruses types 9, 11, and 12.

Burch et al. [23] observed discrete focal interstitial myocarditis in 29 of 50 hearts obtained at autopsy. Using appropriately conjugated specific sera raised against Group B coxsackieviruses, immunofluorescent (IF) myocytes and/or histocytes were found in scattered areas of the myocardia of 12 infants and young children. Other less frequent signals of specific myocardial disease include the rescue of enteroviruses from pericardial fluid and/or myocardial tissues.

Cynomolgus monkeys infected experimentally with coxsackieviruses Group B, type 4, develop pericarditis, myocarditis, and endocarditis; there may be associated injury to valvular leaflets and chordae tendinae [24]. Myocardial and endocardial lesions contain multinucleated giant myocytes similar to Aschoff bodies. Viral antigens were observed by IF within the perinuclear cytoplasm of myocardial fibers.

Myocardiopathies in mice develop after infection from coxsackieviruses [25,26,27]. The kinds of injury vary according to age, sex, and cardiotropic potential of the virus. Group A, type 9, and Group B, type 1, evoke mild focal lesions in adult mice. Group B, types 3 and 4, cause severe injury in weanling mice, expressed as pericarditis and myocardial necrosis worst in the left ventricles and interventricular septa. The visceral pericardium and mural endocardium exhibit advancing fibrosis within the months following infection. Valvular fibrosis has been noted [25]; similar lesions may be observed with advancing age in control mice [27]. There is enduring cardiac hypertrophy and myofibril scarring. Young mice force-exercised had a significantly higher death rate (arrhythmias?) than infected mice not exercised.

The cardiac lesions developing in animal models experimentally infected with coxsackieviruses simulate those observed in fatal infections of human beings. In addition to the acute primary infection from which patients seem to recover, these cardiotropic viruses may provide permanent cardiac injury. Latent damage to the myocardium prepares the way for lingering effects expressed by arrhythmia and cardiac failure.

INSULIN-DEPENDENT DIABETES MELLITUS (IDDM)

IDDM develops in youth (mean age ~ 12 years). The disease evokes loss of beta cells in the islets of Langerhans. Small lymphocytes and scattered polymorphonuclear cells infiltrate the injured islets, mainly in apposition to injured beta cells.

Compelling data [28,29,30] support the concept that viruses can injure the pancreas. Although other viruses [31] may be involved, the concern here relates to coxsackieviruses, particularly Group B strains. Some strains cause profound injury of
exocrine and endocrine tissues of the murine pancreas, especially after consecutive passages of infective pancreatic brei. The B-4 virus grown in cultured pancreatic islet tissue produces injury restricted to beta cells. After selective destruction diabetes develops upon passage of such cells in susceptible strains of mice. Similar findings have been described for the encephalomyocarditis (EMC) virus.

Direct evidence of coxsackievirus-induced IDMM was obtained from a ten-year-old succumbing after an influenza-like illness associated with diabetes. Many beta cells of the pancreas were destroyed. Pancreatic tissue extracts yielded coxsackievirus, B-4. The virus recultured in sensitive islet cells (in vitro) produced diabetes and the pathological sequences noted above in susceptible mice [32].

While there is not a uniform consensus that all IDDMs are caused by viruses, the available evidence indicates strong associations. In tandem with the cellular injury, antibodies are raised against islet cell cytoplasmic and surface membrane components. Cell-mediated antibodies and cytotoxicity develop early after onset. Whether or not these are linked to virus-induced injuries of cellular components is yet unknown. Moreover, the relationship of high risks associated with alleles linked to the HLA complex and virus susceptibility is not clearly defined.

SUMMARY

A library of information has accrued for the human enteroviruses and their association with infection and disease. For most of these viruses there are common but sometimes variable threads of information relating to clinical spectra, pathogenesis, epidemiology, and immunology. This paper cites several common and some of the less common characteristics of enteroviruses in a variety of disease expressions.

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A more detailed bibliography is available on request.