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High Seroprevalence of Neutralizing Capacity against Human Metapneumovirus in All Age Groups Studied in Bonn, Germany

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Human metapneumovirus (hMPV) infections occur frequently despite high rates of perpetual seroprevalence for all age groups. Analyses of ~2,000 archived, randomly selected serum samples demonstrated that neutralizing capacities remain high, with a minor decrease for individuals over 69 years of age, leading to the hypothesis that reinfections occur because humoral immune responses play minor roles in the clearance of hMPV infections.

The human metapneumovirus (hMPV) was first isolated in 2001 from children hospitalized with acute respiratory infections (ARIs) (18). hMPV belongs to the Paramyxoviridae family and is one of the major causes of upper and lower ARIs. By the age of five, nearly every child has been infected with hMPV (18). Since hMPV was first described, it has been reported from all over the world, with prevalences of infection ranging from 3.9 to 43% (12, 13). hMPV seems to have a seasonal distribution, like respiratory syncytial virus (RSV) and influenza virus. Infections occur mainly during the winter months (2, 14, 20). Up to now, two genotypes (A and B), each with two subgroups (A1 and A2 and B1 and B2, respectively), have been identified (11), but it is not known if the two genotypes represent two serotypes and if they lead to variations in the severity of clinical symptoms (19). Symptoms associated with a hMPV infection range from mild infections of the upper respiratory tract to severe lower respiratory tract infections like bronchiolitis and pneumonia. Wheezing, coughing, fever, and dyspnea are frequently observed (2, 9, 18). More-severe hMPV infections primarily affect infants and children, while otherwise healthy adults suffer solely from influenza-like illnesses. However, immunocompromised adults show exacerbated courses of asthma and chronic obstructive pulmonary diseases (8, 10, 21). For the elderly, only a few studies have been released, but it has been stated that hMPV infections often lead to hospitalizations and are associated with high mortality in the elderly (3–5). The aim of the present study was to analyze patient sera for the ability to neutralize hMPV and to investigate whether there are any differences among the different age groups.

Serum samples from a total of 2,000 patients were randomly collected from the archives of the Institute of Virology of the University Hospital Bonn (which includes a large trauma center for the geographic area and a large obstetrics unit, resulting in many patients in the 20- to 50-year-old age range) and screened for neutralizing capacity, using the XTT-based neutralization test described previously (17).

In brief, 5 × 10^4 genome equivalents (geq) of hMPV cells in 50 μl of Dulbecco’s modified Eagle’s medium (DMEM) or 50 μl of DMEM without the virus was applied to the wells of a 96-well plate (Nunc, Karlsruhe, Germany). Afterward, 25 μl of sera was added to each well. Finally, 5 × 10^4 HepG2 cells in 125 μl of medium were added to each well and preincubated for 30 min. The DMEM formulation was clear DMEM with 4.5 g liter⁻¹ glucose, 3% (vol/vol) fetal calf serum (FCS), 1% (vol/vol) 100× penicillin-streptomycin mixture (10,000 U/ml of penicillin and 10 mg/ml of streptomycin), 1% nonessential amino acids, 1% t-glutamine, and 1% sodium pyruvate (all from PAA, Austria).

The cells were incubated for 7 days at 33.4°C and 5.0% CO₂. The confluence and morphology of the cells were controlled daily under an inverse microscope. At day 7, 150 μl of supernatant was removed from each well and discarded. The prewarmed (37°C) XTT test kit solutions were mixed by pipetting the coupling reagent into the yellow tetrazolium salt. Fifty microliters of the solution was added to each well, and the plate was incubated for 1 h at 33.4°C and 5.0% CO₂ before extinction was measured at 456 nm, with 650 nm as the reference measurement, in a 96-well plate reader. For additional verification of the results, cells were counterstained with crystal violet. To investigate the neutralizing capacity of the tested patients’ sera, the results of the XTT test of the cells infected with hMPV and treated with patients’ sera were compared to a reference dilution series and the results for the correspond-
Table 1. Age distribution of patients and number of samples per group that tested positive or negative for neutralizing ability

| Age (years) | Total no. of samples | No. (%) of positive samples | No. (%) of negative samples |
|------------|----------------------|-----------------------------|----------------------------|
| 0–2        | 87                   | 81 (93.1)                   | 6 (6.9)                    |
| 2–9        | 6                    | 6 (100)                     | 0 (0)                      |
| 10–19      | 52                   | 51 (98.08)                  | 1 (1.92)                   |
| 20–29      | 857                  | 831 (96.97)                 | 26 (3.03)                  |
| 30–39      | 435                  | 416 (95.63)                 | 19 (4.37)                  |
| 40–49      | 299                  | 290 (97)                    | 9 (3)                      |
| 50–59      | 142                  | 135 (95.07)                 | 7 (4.93)                   |
| 60–69      | 50                   | 46 (92)                     | 4 (8)                      |
| 70–79      | 20                   | 18 (90)                     | 2 (10)                     |
| 80–89      | 5                    | 5 (100)                     | 0 (0)                      |
| Σ          | 1,953                | 1,879 (96.21)               | 74 (3.79)                  |

* Neutralizing capacity was determined by using an XTT-based neutralization assay.

The results correlate with previous seroprevalence studies, which report that 90 to 100% of children 5 to 10 years old have experienced infection with hMPV (7, 16, 18, 22). In the present study, the 0- to 2-year-old group seemed to have a higher rate of seroprevalence than the >2- to 9-year-old group. This finding may be due to different factors. First of all, the neutralization test does not determine the total amount of hMPV-specific antibodies in a patient’s serum; instead, it identifies the ability of a patient’s serum to neutralize the virus. The sera of very young infants may contain antibodies obtained from breast milk; it can also be assumed that as the sera used in our study were obtained from children hospitalized for different causes, the sera contained immunoglobulins or drugs administered for therapeutical reasons. Although this hypothesis should be tested further with a larger number of patients in the >2- to 9-year-old age group, our study supports it, as we observed a tendency for the neutralization capacity of sera to increase up to the age of 12 months, decrease from 1 to 2 years of age, and then increase gradually up to adulthood (Fig. 1b). Younger children, as well as the elderly, often develop severe courses of diseases after an infection with hMPV, which is surely connected with the efficiency of the immune system, as immunocompromised patients also suffer from severe etiopathologies.

The adaptive immune system develops permanently during aging. Therefore, young children have not yet established a mature immune response, as they have not been in contact with as many pathogens as adults have. After the maturation of the immune system, pathogens can be eliminated faster and more easily. Later in life, in the elderly population, the immune system suffers from a change in immunity, termed immunosenescence. The term immunosenescence encompasses all processes leading to a dysfunctional immunity in the elderly. It is mainly characterized by failures of the T-lymphocyte system (15). These failures manifest in a dislocation of the ratio between cells previously exposed to an antigen and cells able to recognize and attack new antigens to the benefit of the antigen to which the first exposure was experienced (15).

Neutralizing antibodies seem to be present in all age groups, as the results of the present study indicate, so it may be the efficacy of the antibodies or the contribution of such neutralizing antibodies to protection against hMPV reinfections that changes during the human life span. A study in a BALB/c mouse model showed that infectious hMPV persists in the lungs despite the presence of neutralizing antibodies (1). Moreover, antibody depletion of T cells and natural killer cells results in higher titers of hMPV in the lung, further supporting...
the theory that the immune system in the elderly becomes malfunctional.

In another BALB/c mouse study, 8- and 18-month-old mice were infected with hMPV. The aged mice showed a higher level of clinical severity, and the production of virus-specific antibodies and neutralizing antibodies was lower in the aged mice than in the young mice (6). Although this latter observation was for a primary infection in aged mice, it may also be indicative of the situation in the elderly, in which a reinfection is more likely. In concert with the results presented here, this observation leads to the hypothesis that it is the host’s T-cell response that clears hMPV infection rather than the neutralizing humoral response; immunosenescence seems to be characterized not by a lack of protective antibodies but by an insufficient cellular response.

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