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Lack of evidence for an association between hantavirus infections and Wegener’s granulomatosis, microscopic polyangiitis, Churg–Strauss syndrome and giant cell arteritis

Sir. Systemic vasculitides are a heterogeneous group of disorders characterized by inflammation of vessel walls. Much progress has been made in understanding their pathophysiology and pathogenesis, but the cause remains unclear in the majority of the cases. Associations with distinct viral infections have been demonstrated [1]. Among agents that have been implicated are hantavirus, the infectious causes of haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome [2]. HFRS occurs worldwide and may be clinically inapparent. Hantaviruses are known to infect endothelial cells in vitro and in vivo, inducing acute vascular inflammation [3]. Moreover, there is evidence that they can generate the formation of immune complexes [4]. However, it remains obscure whether hantavirus infections are involved in the development of chronic vascular inflammation. We therefore investigated the prevalence of hantavirus-specific antibodies in a large group of patients with well-defined forms of systemic vasculitides.

The sera studied were collected from patients attending the Departments of Rheumatology in Bad Bramstedt (Rheumaklinik) and Lübeck (University of Lübeck), Germany, between 1994 and 1998. These were 101 patients with Wegener’s granulomatosis (WG) and 30 patients with microscopic polyangiitis (MPA), Churg–Strauss syndrome (CSS) and giant cell arteritis (GCA), classified according to the definitions of the Chapel Hill Conference and the classification criteria of the American College of Rheumatology [5, 6]. Control sera were obtained from 69 patients with rheumatoid arthritis (RA) who fulfilled the American College of Rheumatology criteria [7], and from 238 healthy blood donors (Table 1). Patients and controls were resident in northern Germany. Antineutrophil cytoplasmic antibody titres >1:8 determined by indirect immunofluorescence were regarded as positive.

The enzyme-linked immunosorbent assay (ELISA) for the detection of hantavirus-specific IgG antibodies was performed as described previously [8]. Briefly, microtitre plates (Nalge Nunc International, Albertslund, Denmark) were coated overnight at 4°C with a basic buffer detergent extract of Puumala or Hantaan-infected Vero cells inactivated with 2 × 10⁶ rad from a ⁶⁰Co source. Standard methods were followed, and uninfected antigen controls were run for each serum. Subsequently, wells were incubated with human serum followed by a polyclonal antibody against human IgG conjugated to horseradish peroxidase (Dako, Hamburg, Germany), each for 1 h at 37°C. Substrate solution (Kirkegaard & Perry Laboratories, Gaithersburg, USA) was added for 30 min at 37°C and signals were measured by spectrophotometry [optical density at 410 nm (OD₄₁₀)]. To yield the adjusted OD (adOD) value, the reading of the negative control antigen was subtracted. The cut-off was defined as adOD 0.100. All sera reacting positively in a 1:100 dilution were tested again in a twofold serial dilution. Serum titres ≥ 1:400 were considered as seropositive if the added adOD₄₁₀ values of positively reacting serum dilutions exceeded 1.0.

A total of 191 sera obtained from patients with different forms of systemic vasculitides were tested,

| Table 1. Demographic data and hantavirus screening of patients and blood donors |
|-------------------------------|---------------------|---------------------|-----------------|---------------------|
|                              | n       | Female (%) | Age range (yr) | Mean age (yr) | ANCA positivity |
|                              |         |            |                |               | cANCA | pANCA |
| Blood donors                 | 238     | 46.6       | 18–74          | 32.0          | NT    | NT    |
| Rheumatoid arthritis         | 69      | 72.5       | 21–90          | 57.0          | 0     | 2     |
| Wegener’s granulomatosis     | 101     | 50.5       | 26–77          | 55.0          | 75    | 2     |
| Microscopic polyangiitis     | 30      | 60.0       | 17–86          | 59.0          | 0     | 16    |
| Churg–Strauss syndrome       | 30      | 53.3       | 22–70          | 43.0          | 2     | 0     |
| Giant cell arteritis         | 30      | 90.0       | 41–80          | 66.5          | 0     | 0     |

NT, not tested.

ANCA, antineutrophil cytoplasmic antibodies.
including 30 from patients with localized WG. None of them displayed IgG antibodies to Puimala or Hantaan viruses. This was also true for 69 sera from patients with RA and 238 sera from healthy blood donors (Table 1). Control measurements of sera from patients with clinically apparent Puimala or Hantaan infections confirmed the validity of the assay used.

To the best of our knowledge, this is the first comprehensive study investigating the prevalence of hantavirus-specific antibodies in patients with systemic vasculitides. In our well-defined population of 191 patients with WG, MPA, CSS and GCA, we found no serological evidence of an association between hantavirus infections and the development of systemic vasculitides. The lack of positive sera among patients and controls suggests a low prevalence of hantavirus infections in the northern German population in general. However, the specific rodent reservoirs (Clethrionomys glareolus, Apodemus agrarius) are present and clinical cases of acute hantavirus infections (Puimala and Dobrava) are seen at the Lübeck University Hospital.

Antibody titres may decrease after the infection has resolved. Thus, we might have missed previous hantavirus infections in some cases. To minimize this bias we investigated sera from patients with short-term as well as those with longstanding disease. The lack of positive cases in both groups suggests that a time-dependent decrease in antibody titres was not a major problem. Moreover, previous studies have shown that elevated antibodies to hantaviruses generally persist for many years [9].

Our findings contrast with the results of a recent small uncontrolled study conducted in Bavaria, Germany. Among 26 patients with WG or MPA, Hierl et al. [10] detected antibodies to hantavirus in 15%. This high prevalence may be explained by their use of an immunofluorescence assay (Progen, Heidelberg, Germany), while we performed an internationally recognized ELISA [8]. Moreover, Hierl et al. used a cut-off titre of 1:16, resulting in relatively low specificity. In fact, none of their patients had a hantavirus antibody titre above 1:32.

In summary, our data indicate that hantavirus infections are not associated with the development of WG, MPA, CCS or GCA. Serological or molecular approaches might identify other infective agents in rheumatological disorders in the future.

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