The authors of several studies have observed increased intraalveolar cytokine production *(19)* or increased CD4+ T lymphocytes in smokers *(20)*. A suppressive effect of tobacco smoke on the human immune system has also been reported *(21)*, which could explain the lower neopterin production of smokers in our study.

We noted a correlation between neopterin concentrations and diastolic blood pressure values. Although an explanation for this correlation is not immediately evident, the possibility of a connection between immunoactivation and increased diastolic pressure cannot be excluded definitively. Recently, a correlation was reported between ischemic attack and both neopterin and the potent endothelial-derived, vasoconstrictive peptide endothelin-1 *(22)*. Conditions such as atherosclerosis *(16)* or infections by *Helicobacter pylori (23)*, which are associated with immune activation and higher neopterin production, might possibly lead to vasoconstriction and thereby increased diastolic blood pressure because of increased endothelin-1 production.

The positive correlation that was found between neopterin concentrations and BMI, a correlation that was not an independent one, supports results of an earlier study in individuals with non-insulin-dependent diabetes mellitus *(13)*, and it would be in line with earlier suggestions that long-lasting immune activation, even if only moderate, may contribute to the development of obesity *(24)*.

Nevertheless, our study of blood donors indicates that neopterin concentrations are associated with age, smoking status, diastolic blood pressure, and BMI, although the reasons for these associations are still unclear. The interindividual variation of neopterin concentrations among healthy individuals may, in part, be attributable to variations caused by these variables rather than analytical variation of the assay used.

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Serologic Diagnosis of Hantaan Virus Infection Based on a Peptide Antigen, *Zheng Li,[1] Xuefan Bai,[2] and Huijie Bian[3*]* ([1] School of Chemistry and Material Science, Shaanxi Normal University, 710062, Xi'an, China; [2] Department of Infectious Disease, Tangdu Hospital, [3] Cell Engineering Research Centre, Fourth Military Medical University, 710032, Xi'an, China; * author for correspondence: fax 86-29-3293906, e-mail bhj@pub.xaonline.com)

Hantavirus (HV) is the causative agent of a severe type of hemorrhagic fever with renal syndrome (HFRS), with an annual incidence in China of 50 000–100 000 cases. Hantaan virus (HTNV) 76–118 is the prototype strain of the HV genus and the hantaan serotype *(1)*. HV has a single-stranded, negative-sense tripartite RNA genome, the segments of which are designated large, medium, and small. The RNA genome encodes the viral RNA polymerase, envelope glycoproteins (G_1, G_2), and nucleocapsid protein (NP) *(2)*. A serologic investigation *(3)* showed that HTNV NP has strong antigenicity and immunogenic-

ity, and the antibodies against NP in patients with HFRS not only appear early, but also have high titer. Moreover, NP contains the major antigen that can cross-react with the immunized sera of many serologically and genetically distinct groups of HV *(4)*. To screen the epitopes of NP with monoclonal antibodies against HTNV, a library of HTNV small gene-peptide fragments on phages was constructed. Results showed that a linear epitope consisting of amino acids 1–86 (aa 1–86) within the NP reading frame was determined and its core sequence consists of aa 15–66, which is a major and cross-reactive antigenic epitope *(5)*.

Earlier serologic assays for the detection of HTNV-specific immune responses were based on virus propagated in cell culture *(6)*. The hazardous nature of HTNV, its slow replication, and low yield in cell culture have prompted expression of recombinant HTNV NP for use as an antigen in ELISA *(7)*. However, the expression and purification of recombinant HTNV NP is not straightforward for either the full-length or partial recombinant HTNV NP *(8)*. It is necessary to establish a novel specific serologic diagnosis of HTNV infection.

We developed an ELISA-based method for the detection of HFRS, using a synthetic peptide antigen. Synthetic peptides that mimic specific epitopes of infectious viral proteins have been used in diagnostic systems for various diseases and for the molecular design of vaccines *(9, 10)*. Synthetic peptides offer the advantage of eliminating nonspecific reactions resulting from the cross-reactions of antibodies in the specimen with host-cell-derived viruses or recombinant products derived from *Escherichia coli (11)*.

For computer-assisted epitope prediction, we used the software Antheprot V4.3c (http://pbil@ibcp.fr) and predicted hydrophobicity and antigenicity, together with predictions of α, β, turn, and coil conformation probabilities. This indicated that the most probable antigenic site of HTNV NP is between aa 17 and aa 66 with the NH_2 terminus reading frame. Then the peptide (aa 17–66) of HTNV NP was synthesized by solid-phase peptide synthesis. The crude peptide was first purified by a G-25 column. The preparation and analysis of the peptide was then performed with reversed-phase HPLC. The results from amino acid analysis were as follows: $Thr_{1.9}Ser_{1.03}Ala_{3.82}Asp_{5.03}Glu_{5.02}Gly_1Ile_{2.86}Lys_{6.86}Leu_{5.03}Asn_{1.02}Pro_{1.03}Gln_5Arg_{5.04}Val_4Tyr_{0.9}$.

Micro-ELISA plates were coated with the synthetic peptide in 0.02 mol/L Tris-HCl buffer (pH 8.0) for 12 h at 37°C. The peptides were used in a free form. Free binding sites were saturated by incubation with 30 g/L bovine serum albumin in phosphate-buffered saline (PBS). After 1 h of incubation at 37 °C, the wells were washed four times with PBS-Tween 20 (PBST). Sera were diluted 1:100 (1 volume of serum + 99 volumes of PBST) in PBST with normal goat serum (200 mL/L) containing Triton X-100 (5 mL/L). The mixture was incubated for 1 h at 37 °C. For IgM detection, the sera were first treated with 10 μL of goat antibodies to human IgG to remove the IgG. After washing, horseradish peroxidase (HRP)-conjugated goat antibodies to human IgG or IgM diluted 1000-fold in PBST containing 10 g/L bovine serum albumin was added for 1 h. The wells were washed four times with PBST, and the bound HRP label was detected with 3,3′,5,5′-tetramethylbenzidine as the substrate for 30 min in the dark, after which time the color reaction was stopped by the addition of 2 mol/L H_2SO_4. The cutoff for positive was defined as the mean absorbance (+3 SD) at 492 nm for 81 control sera; the cutoff value was 0.070.

Molecular characterization of the IgM and IgG immune responses against HRFS was performed by analysis of serum samples. The synthetic peptide was applied as an antigen to test the specific antibody (IgM, IgG) by ELISA. Forty-six sera from HFRS patients, 9 sera from HBV patients, and 81 sera from healthy people were analyzed. The samples from healthy volunteers were analyzed in the same run as the HFRS samples. The analyst knew which samples were from which group (HRFS, HBV, and healthy volunteers). In this preliminary study, the sera of the HBV patients and healthy individuals were negative for anti-aa 17–66 IgM, and 4 sera of healthy people were positive for anti-aa 17–66 IgG. Among HFRS patients, 43 of 46 sera were positive for anti-aa 17–66 IgM (Fig. 1A), and 42 of 46 sera were positive for anti-aa 17–66 IgG (Fig.

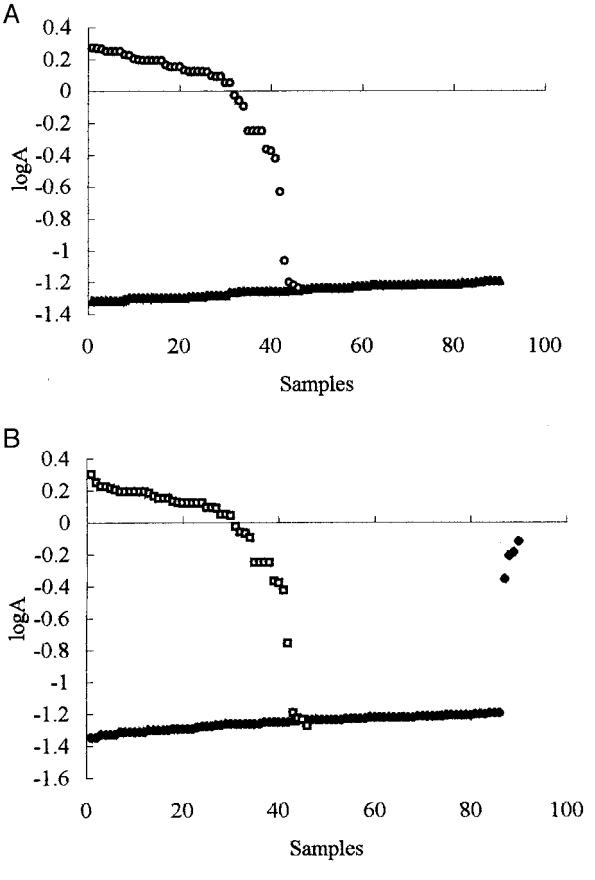

Fig. 1. ELISA results for patients with (*open symbols*) and without (*closed symbols*) HTNV virus infection.

(*A*), IgM detection. (*B*), IgG detection. *Vertical axes* represent log of absorbance; *horizontal axes* represent sample number.

1B). Future clinical studies should be performed to better establish the sensitivity and specificity of this assay.

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Maternal Nitric Oxide Supplementation Decreases Cord Blood S100B in Intrauterine Growth-retarded Fetuses,

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Intrauterine growth retardation (IUGR) is thought to reflect suppression of genetic growth potential by decreased supplies of oxygen and substrate *(1)*. NO supplementation may be useful in IUGR to increase uteroplacental circulation *(2–5)*. The use of biochemical markers to assess the extent of brain distress associated with NO treatment could be appropriate. S100B is an acidic calcium-binding protein of the EF-hand family concentrated in the nervous system *(6)*. The appearance of S100B in biological fluids is an indicator of brain distress in both infants and adults *(7–10)*. Recently, blood S100B concentrations in the perinatal period have been shown to correlate with brain maturation and damage *(11–14)*.

We investigated the effect of maternal NO supplementation on brain distress in IUGR fetuses as assessed by cord blood S100B. We selected 51 pregnant women (gestational age, 27–35 weeks) with IUGR fetuses and impaired uteroplacental blood flow. Exclusion criteria included multiple pregnancies, gestational and type 1 diabetes, maternal infections and fever, fetal malformations and chromosomal abnormalities, metabolic diseases, and maternal diseases of the central nervous system. Patients were assigned, by use of computer-generated random numbers, to receive either placebo (n = 25) or transdermal glyceryl trinitrate (Nitroderm TTS; Ciba-Geigy) at a dose of 5 mg/16 h daily until delivery (n = 26). Placebo and glyceryl trinitrate patches were indistinguishable and were numbered and contained in identical envelopes so that patients did not know the group to which they were recruited. Similarly, neither the physicians nor the investigators who analyzed the samples knew which patients were treated with placebo and which with glyceryl trinitrate patches. The local ethics committee approved the study, and written informed consent was obtained from all participants.

Two patients in the glyceryl trinitrate group declined to accept administration of the drug, and one did not follow correctly the indications for patch application and was removed from the data analysis.

Gestational age at the time of enrollment did not differ between the two groups. All fetuses were delivered by elective cesarean section for obstetrical reasons and did not experience uterine contractility. Gestational ages were determined by clinical data and by a first-trimester ultrasound scan. IUGR was defined by the presence of ultrasonographic signs (biparietal diameter below the 10th centile and abdominal circumference below the 5th percentile) according to the normograms of Campbell and Thoms *(15)* and by a fall in the centile of fetal size recorded between the first scan after referral and subsequent scans. The flow velocity waveforms (FVWs) of the main branch of the uterine artery bilaterally, the umbilical artery (UA), and fetal middle cerebral artery (MCA) were recorded by means of a duplex pulsed color Doppler ultrasound (SSD-2000; Aloka). A reading >2 SD above the mean for the gestational age in uncomplicated pregnancies was defined as a pathologic resistance index (RI) for the uterine artery and as a pathologic pulsatility index (PI) for the UA. An abnormal PI in the FVWs of the fetal MCA was defined as a pathologic RI for −2 SD from the mean for that particular gestational age in uncomplicated pregnancies. A UA/MCA PI ratio >1 was considered patho-