Neopterin, β_2 -Microglobulin and Carbohydrate Antigen CA-19-9 in Sporadic Adult Amyotrophic Lateral Sclerosis

Martin E. Westarp^{1§}, Dietmar Fuchs², Peter Bartmann³, Rikke Hoff-Jørgensen⁴, Jörgen Clausen⁵, Henrik Rasmussen⁵, Helmut Wachter², Maria P. Westarp¹ and Hans-Helmut Korhnuber¹

¹Ulm University Department Neurology, RKU, Oberer Eselsberg 45 D-89081 Ulm, Germany

²Institute for Medical Chemistry and Biochemistry, and Ludwig-Boltzmann Institute for AIDS Research, A-6020 Innsbruck/Austria

³Ulm University Department Pediatrics, Prittwitzstr. 43, D-89075 Ulm, Germany

⁴National Veterinary Laboratory, 27 Bülowsvej, DK-1790 Copenhagen, Denmark

⁵Institute for Life Sciences and Chemistry, POB 260, DK-4000 Roskilde, Denmark

(Received February 28, 1993)

Summary

In 38 patients diagnosed as adult sporadic amyotrophic lateral sclerosis (ALS), and negative for relevant anti-ganglioside, anti-borrelia or anti-Borna disease virus antibodies, we determined serum concentrations of neopterin, β_2 -microglobulin, immunoglobulin-G isotypes G₁-G₄, carbohydrate antigen CA-19-9, as well as repeated enzyme linked immuno-assay (ELISA) serum antibodies to human spuma retrovirus (HSRV, human foamy virus=HFV) envelope plus gag/capsid antigen, and competition with maedi-visna virus antibodies in an blocking ELISA. Confirmed by specific HFV-gag immunoblots and measured under code, 26% of tested ALS-patients and 4% of 195 other neurological controls reacted ELISA and immunoblot antibody-positive against HFV. In a maedi-visna virus blocking ELISA, 47/50 sera from 28/30 ALS patients competed with anti-visna lentivirus antibodies more than healthy controls ($p < 0.05$). In serum, however, β_2 -microglobulin and neopterin concentrations were normal in different stages of motor neuron disease. Maximal individual mean values for serum CA-19-9, anti-visna competition or circulating IgG immune complexes associated with positive serum antibodies against human recombinant foamy retroviral antigen, while macrophage activation measured as serum neopterin did not.

Key words: Amyotrophic lateral sclerosis, β_2 -microglobulin, Carbohydrate antigen CA-19-9, Human endogenous retroviral sequences (HERV), Human spuma retrovirus (HSRV), Immunoglobulin-G isotypes, Neopterin

Introduction

Human foamy retrovirus has become a prime candidate pathogen for neurodegenerative central nervous motor disorders (1), since transgenic expression is brain- and muscle-specific (2, 3), and mice predominantly develop myatrophy and encephalopathy (4). Recently (5, 6), we reported on an upregulated humoral immune response against human spuma retrovirus, a representative of the still incompletely

characterized foamy retroviruses (7), in patients with idiopathic ALS. With documented elevated circulating immune complexes (8), cerebral lesions in magnetic resonance imaging (9), an inverse correlation between age and duration as in HIV infection (10), an elevated lymphoma incidence (11) and a monophasic progressive clinical course, ALS shares partial analogies with other retroviral diseases (12, 13). In adult sporadic motoneuron disease, increased incidences have been reported for anti-retroviral and anti-ganglioside GM₁ antibodies; activated macro-

[§]Author to whom correspondence should be addressed.

phages have been found infiltrating the spinal cord in ALS (14). While in ALS T-lymphocyte subpopulations were normal (11, 13, 14), β_2 -microglobulin and neopterin as markers of a cellular immune response have not been documented; in autoimmune disorders neopterin levels correlate with extent and activity of the disease (15). Neopterin and β_2 -microglobulin are regularly increased in human immunodeficiency virus type-1 (HIV-1) infection, elevated in a proportion of human T-lymphotropic retrovirus (HTLV-1)-positive neurological patients (16), yet unchanged in Parkinson's disease (17). D-erythro-neopterin is produced by stimulated macrophages, and correlates well with in-vitro replicative capacity of HIV-1 isolates (18); β_2 -microglobulin constitutes the light chain of major histocompatibility complex (MHC) class I antigens HLA-A, -B and -C, identical with thymotaxin, and present in high densities on lymphocytes. In this paper, we examine anti-retroviral antibodies, neopterin, β_2 -microglobulin, carbohydrate antigen CA-19-9 and serum immunoglobulin isotypes in ALS. Anti-HFV seronegativity correlates with low IgG₃ plasma concentrations, while anti-HFV seropositivity associates with increased binding of ALS sera to maedi-visna antigen or elevated levels of circulating asialo-ganglioside CA-19-9, respectively.

Patients and Methods

Human foamy virus (HFV)=spuma retroviral (HSRV) antibodies were determined in 38 ALS patients, 396 non-ALS patients and a total of 600 coded sera as described in (1, 19) using identical recombinant HFV envPX envelope and gagCA capsid antigens (20); 195 Ulm neurological in-patients without acute cranial nerve affection, myelopathy or Parkinson's syndromes served as controls ("other neurological diseases"). Diagnostic criteria for 38 patients with definite idiopathic ALS (aged 19-78 years, mean age of 58.4 years) were signs of lower motor neuron involvement (fasciculations, progressive muscular wasting and weakness in the absence of progressive sensory deficits and multifocal conduction block), involvement of upper motor neuron at some stage of the disease, and possible development of bulbar symptoms and signs. No patient was suffering from recurrent or concomitant infectious diseases, including hepatitis, borreliosis or acute herpes infection, all 24 consenting patients examined were negative for antibodies to human immune deficiency virus. All patients had areactive treponema pallidum hemagglutination tests, were negative for monoclonal

gammopathy, relevant anti-glycolipid antibodies to GM-1, GD-1b, GA-1, 3'-LM1 and sulfatide, separately tested anti-GM1 IgM antibody titers, or IgG ELISA antibodies to Borna disease virus (21). A maedi-visna lentivirus competition ELISA was done in 50 sera available unfrozen from 30 ALS patients aged 19-78 years and tested for HFV antibodies within three weeks. Blocking of specific polyclonal anti-visna antibodies was expressed as reduction in binding after pre-incubating visna antigen with patient sera, and standardizing blocking ELISA endpoint optical densities as described (22, 23) to values established for healthy Danish adults. Results in % reduction were compared with 28 age and sex matched patient controls (not tested for HFV) as well as 15 "other neurologic diseases" tested HFV-seronegative. Circulating IgG immune complexes (CIC) were quantitated by standard Raji cell assay (healthy references <2.4 mg/L, patient normal reference limit 14 mg/L); sera had been frozen immediately.

Results

Foamy retrovirus

Antibody seropositivity for human foamy retroviral (HFV, human spuma retrovirus) antibodies was a significant finding in idiopathic ALS ($p < 0.01$). Examined consecutively and under code, 18/38 ALS patients reacted HFV-env and/or HFV-gag ELISA antibody positive compared with 33/195 neurological controls and 7/52 orthopedic patients (Fig. 1). In contrast to only 8/195 (4%) of "other neurological controls", 26% of ALS patients could be confirmed seropositive for anti-HFV-gag antibodies by immunoblot. Over the last 24 months, 18/38 ALS patients (45%) and 34/195 other neurological controls (18%) had reacted either ELISA or immunoblot HFV antibody positive. Seropositivity was independent of duration of disease, age, circulating immune complexes, total cerebro-spinal fluid protein, course or variant of motor neuron disease. Of 35 patients who recalled the onset of first signs or symptoms by month, 9 HFV-positive and 9 HFV-negative patients reported a clinical onset in their upper extremities, 7 HFV-positive and 5 HFV-negative patients in their legs or feet, and 1 HFV-positive and 4 HFV-negative patients a (pseudo-) bulbar onset of the disease. Mean age at first clinical symptoms was 59.7 years for primary bulbar disease, 56.7 years for start in the upper extremities, and 53.6 years for begin in the legs (see Table 1). Two seropositive idiopathic ALS patients had been treated for sporadic "essential tremor"

Human foamy retrovirus antibodies

Figure 1. Significantly elevated anti HFV-env and/or HFV-gag ELISA and anti HSRV-gag immunoblot antibodies in ALS:

Summary of ELISA and immunoblot screening for human foamy virus antibodies in diagnostic subgroups larger than 30 patients; ALS=amyotrophic lateral sclerosis, Park.=Parkinson's syndromes, MedPer=hospital personnel, OND=other neurological diseases as defined (cf. methods), Ortho=orthopedic patients Ulm 1990-1992. SurgPat=South-West German surgical patients reported earlier (1) and tested in the same laboratory 1989-1991.

with oral propranolol for over 12 months prior to progressing to ALS. On cerebral nuclear magnetic resonance studies 5/7 anti-HFV-positive and 1/4 anti-HFV-negative motor neuron disease patients had native magnetic resonance scans unremarkable for age. Small demyelinated asymmetrical lesions in the subcortical layers were seen in three men (HFV-negative 51 y; HFV-positive 55 y, 62 y), whereas left-sided fronto-subcortical, corticospinal tract and rostro-external capsule lesions were seen in one woman with paretic right arm, and multiple bilateral periventricular and left-sided basal ganglia lesions in a woman with tetraparetic begin of the disease (both women aged 58 years, HFV-seronegative).

Visna lentivirus

On ELISA binding, 28 ALS sera from 23 patients competed with anti maedi-visna virus antibodies better than 28 age and sex matched geographic control patients; after pre-incubating ELISA visna antigen with neurologic sera, binding of polyclonal anti-visna antibodies expressed as relative optical density calculated against healthy Danish controls (= 100

Figure 2. Competition by ALS sera with polyclonal maedi-visna virus antibodies (blocking ELISA):

Reduction in optical density (ELISA) by pre-incubation with ALS sera (in %, standardized to healthy controls=0%) as parameter for competition of ALS sera with specific polyclonal anti-visna antibodies. 47/50 sera of 28/30 ALS patients reduce binding of secondary anti-visna antibody. Competition is independent from age at onset or variant of the disease.

%) was $89.0\% \pm 1.9$ (SE) for ALS sera and $93.1\% \pm 1.0$ (SE) for age and sex matched Ulm control patients ($P_{\text{Wilcoxon}} < 0.05$). Expanding the immuno-assay to 50 sera from 30 ALS patients (age 34-78 years), and standardizing to previously established values of normal Danish adults, 12 HFV-positive ALS patients demonstrated a reduction of anti-visna antibody binding by $-14\% (\pm 1.3\% \text{ SE})$, HFV-negative patients by -9.2% ($p < 0.05$), and 15 HFV-negative "acute other neurologic controls" by mean -5.7% ($p < 0.01$ vs. HFV-positive ALS). Pre-incubation with 47 of 50 sera led to -4% to -21% reduction with specific anti-maedi visna antibody binding (Table 1, Fig. 2), and all patients measured more than once remained concordant regarding competition (28/30) or non-competition (2/30). Visna reactivity tended to be more pronounced in sera with high maximal circulating immune complexes (see Table 1). In 10 patients with elevated maximal immunoglobulin-G immune complexes, mean reduction of visna antibody binding was $-13.0\% \pm 1.5 \text{ SE}$, compared with 16 patients with immune complexes $< 14 \text{ mg/L}$ and a mean reduction in visna antibody binding of $-8.8\% \pm 1.5 \text{ SE}$.

Neopterin and β_2 -microglobulin

Neopterin and β_2 -microglobulin concentrations were determined simultaneously in 55 sera and in nine CSF samples of 28 ALS patients, with total CSF protein concentrations ranging from 248 to 803 mg/L. Monocytopenia or neutropenia as an indirect cause for altered neopterin measurements could be excluded in our patients. Three ALS patients aged 62 to 75 years exceeded the all-age neopterin referen-

Table 1. 38 patients with idiopathic ALS:

sec age ¹	clin start ²	anti HFV ³	anti visna ⁴	CSF ⁵ prot	serum ⁶ IgG-3	IgG ⁷ CIC	survival ⁸ months	β_2 M ⁹	neop ¹⁰	CA- ¹¹ 19-9
f28	4L	— (N)			43		>44	1.1	3.6	0
f30	9f	+	— 17%	337	.29		>75			62
f31	10h	+	— 21%	270			>54	1.4	4.3	0
f49	3bu	—	— 14%		.22	+	18	1.6	5.1	0
f53	10s	—	— 4%	336	.25	+	>70	1.3	5.1	8
f55	3a	—	— 5%		.38	—	>70	2.0		0
f55	3bu	— (O)	— 7%	276		++	33			
f55	10A	+++	— 8%	404*		+++	64	1.1	6.7	21
f60	7H	++	— 13.5%*	531		+	42	1.4	6.3	0
f60	12H	—	— 4%			+	70	1.8	6.9	0
f64	11H	—	— 12%	500	.16	+	50	1.6	7.0	0
f67	9f	—		536	.53	—	>50	1.7		8.1
f73	1bu	+++	— 19%	443	.44*	+++	39	1.9	8.2	15
f77	1A	— (C)	— 4%			+	12	2.2	8.5	0
m18	3L	+++	— 11.5%*	248	.37	+++	>74	1.4	5.4	0
m33	—L	—			.20*	—	>100	1.4	4.4	6
m35	9h	—		505	.21*	+	>24	1.2	5.1	0
m37	—L	(+)	— 9%*		.13	++	>192	1.8	5.3	0
m39		—	— 6%				>228			
m39	11	—	— 6%				10			
m43	8bu	—	— 10%	467	.50	+	25	1.7	6.1	5
m46	3s	+++	— 11%		.31		>24	1.4	5.9	0
m50	8a	+	+ 4.5%*	480	.34	+	>50	1.5	7.8	
m54	1s	—	— 17.5%*	300	.27	+++	37	1.2	5.5	12
m54	7A	+++	— 8%			—	20			
m55	10A	+	— 9.5%*		.42	+++	>89			
m56	61	+	— 12.5%*		.37	++	90	1.3	3.7	80
m58	7AL	+ **			.48*	—	12	1.9	6.9	7
m60	3h	+++	— 18.5%*	602*	.98	+++	45	2.3	8.2	0
m60	121	—		484	.15	++	15	1.7	7.2	0
m61	4a	—	— 7%			—	>80			
m63	7f	—	— 5%*	500	.28	+	74	1.6	5.0	16
m65	1L	+	— 18.5%*	595	.43	+	34	1.5	4.8	9
m66	12a	++	— 16%	658	.36	+	15	1.5	6.6	
m67	5L	+ (E)		381	.18	—	>12	2.8		0
m69	2bu	— (N)	— 17.5%*		.13	++	23	1.7	5.9	0
m69	21	—	— 15.5%*	803	.24	—	31	1.5	6.1	0
m70	3L	+	— 9%	662		—	>33	2.6	10.1	149
		18/38 ELISA pos.	30/31 reduce binding			20/27 <.4 g/L IgG ₃	23/32 CICs de- tectable			
								27/28 neopt. <8.8 nM		

1) sex and age in years at onset of the disease, [O=cerebral lesions in magnetic resonance imaging plus autochthonous oligoclonal IgG banding in cerebro-spinal fluid; N=acute neurologic disease within two years before symptoms of ALS, i.e. torticollis (f. aged 28 years) and temporal seizures (m. aged 69 years); C=i.m. injections of homogenized calf spinal cord and cortex by non-medical practitioner prior to first symptoms of ALS (f. aged 77 years); E=multilocular echinococcosis diagnosed four years before ALS (m. aged 67 years)]; 2) site of first clinical deficits (f=foot, f=feet, l=leg, L=legs, a=arm, A=arms, h=hand, H=hands, s=shoulder, S=shoulders, bu=bulbar or pseudobulbar); 3) +++=HFV-env plus HFV-gag antibodies at more than one measurement, ++=more than one env and/or gag antibody-positive measurement, +=confirmed env or gag antibodies at one measurement; bold=anti-HFV-gag capsid antigen immunoblot positive; 4) reduction of secondary anti-visna antibody binding compared with healthy controls (= 100%); 5) cerebro-spinal fluid total protein in mg/L; 6) immunoglobulin IgG₃ isotype serum concentration in g/L; 7) maximal circulating immune complexes +++=CIC>50 mg/L, ++=50 mg/L≥CIC>14 mg/L, +=14 mg/L≥CIC>2.5 mg/L; 8) months between first signs or symptoms and death (or last contact if still alive); 9) β_2 -microglobulin serum concentration in mg/L; 10) neopterin serum concentration in nmol/L; 11) serum CA-19-9 in U/ml (RIA); *) mean of two independent measurements.

Figure 3. Serum β_2 -microglobulin and neopterin in 55 ALS sera:

In 55 sera from 28 ALS-patients (14 HFV-seropositive, 14 HSRV-seronegative), concentrations of the two macrophage activation markers β_2 -microglobulin and neopterin correlated positively ($r=0.7$, $p<0.05$). More CSF measurements are needed.

ce (≤ 8.7 nmol/L), measuring 9.2, 9.3, and 9.5 nmol/L for neopterin, and 2.6, 2.2 and 0.6 mg/L for β_2 -microglobulin. Using age-adjusted reference limits for β_2 -microglobulin (≤ 2.5 mg/L at age <60 years, and <3.2 mg/L at age >60 years), all ALS patients tested within normal ranges (Fig. 3). ALS patients with simultaneously high anti-HFV-env and -gag antibody titers did not differ in neopterin levels (Fig. 3b). CSF concentrations of both β_2 -microglobulin and neopterin never exceeded corresponding serum levels. Including seven moribund and severely affected patients, we could not demonstrate a definite elevation of β_2 -microglobulin and neopterin in ALS; both serum concentrations correlated well ($r=0.7$, $p<0.01$). In contrast, two repeatedly HFV ELISA-positive neurological control patients demonstrated highly elevated values of neopterin (19.4 and 50.5 nmol/L) and β_2 -microglobulin (7.0 and 9.2 mg/L); one of them (a 69-year-old man) was suffering from thyroiditis, an atypical Parkinson's syndrome and incapacitating tongue tremor, the other (a 61-year-old woman) from repeated cerebral microinfarctions with positive anti-nuclear serum antibodies (titer 1:160 and 1:320).

Immunoglobulin isotypes

Twelve sera from ALS patients positive for anti-

HFV antibodies (mean age 55.9 years, range 19-75 years) had a higher mean IgG₃ concentration (47 mg/dl ± 11 SE, $P_{\text{Wilcoxon}} < 0.05$) than 15 sera from anti-HFV negative patients (24 mg/dl ± 2 SE; mean age 52.4 years, range 30-71). Immunoglobulins IgA, IgM and IgG₁₋₄ did not differ between men and women with ALS. Mean values ($n=58$) were IgA=2.6 g/L, IgM=1.2 g/L, IgG=10.9 g/L, and IgG₁=54% IgG, IgG₂=36% IgG, IgG₃=3.7% IgG, IgG₄=6.3% IgG. Most frequent alterations were low total IgG₃ and low total IgG₁. Immunoglobulin reference limits were rarely exceeded, and this never concerned total IgA, IgM, IgG, and IgG₁ or IgG₄. Immunoglobulin isotypes did not correlate with β_2 -microglobulin, neopterin, CA-19-9 circulating immune complexes or serum creatine kinase.

Carbohydrate antigen CA-19-9

We compared CA-19-9 concentrations in 60 ALS sera from 15 HFV-seropositive and 20 HFV-seronegative patients, and in 10 other neurological sera from 7 HFV-seropositive patients. HFV-seronegative motoneuron patients showed a CA-19-9 distribution similar to 1539 healthy controls, and HFV-seropositive motoneuron sera ($n=26$) rather approximated the results from 308 patients with non-malignant gastro-intestinal disorders (Fig. 4, 26). In both HFV-seropositive ALS and HFV-seropositive other neurological patients, CA-19-9 concentrations tended towards higher carbohydrate levels. Three ALS patients exceeded the upper reference limit of 37 U/ml, two neurological control patients with Parkinsonian syndromes reproducibly demonstrated CA-19-9 levels between 23 and 34 U/ml. Individual mean concentrations of six ALS patients exceeded the reference range for healthy individuals (14 U/ml), only one of whom had never demonstrated anti-HFV antibodies in serum. In three of the HFV-seropositive ALS patients with elevated CA-19-9, no significant gastro-intestinal pathology could be documented including abdominal computerized tomography. All eight patients demonstrating either more than 17.5% visna competition by 17.5% or CA-19-9 levels above 17.5 U/ml had tested HFV antibody positive (Fig. 5). Among ten patients without detectable CA-19-9, six had reacted HFV antibody negative ($p<0.01$, Fisher's exact test, Tables 2 and 3).

Discussion

More than one third of ALS sera and patients tested had antibodies to human spuma retrovirus

Figure 4. CA-19-9 in HFV-seropositive patients resembles benign gastro-intestinal disease:

Distribution of carbohydrate antigen 19-9 (asialo-ganglioside) in serum of 34 HFV-antibody negative ALS, 26 HFV-antibody positive ALS, and 10 anti-HFV antibody positive other neurological patients, compared to published controls (26)

HSRV-capsid (gag) and/or envelope (env) antigens which are located on different sites of the proviral DNA. Seropositivity was not confined to motor neuron disease, yet significantly more frequent than in 195 control patients. Standardized to healthy individuals, ALS sera competed with secondary visna-antibodies binding on maedi-visna-viral antigen ($p <$

Figure 5. High CA-19-9 and visna antibody competition in HFV-seropositive ALS patients:

Asialo-ganglioside CA-19-9 in serum, and competition with visna antibodies by ALS sera is maximal in HFV-seropositive patients (8/8 sera CA-19-9 > 17.5 U/ml or reduction of visna antibody binding > 17.5% have tested HFV-seropositive), though carbohydrate antigen and maedi-visna retroviral competition are not correlated ($n = 23$).

Table 2. Higher serum Ca-19-9 in patients seroreactive to HFV:

serum CA-19-9	≤ 17.5 U/ml	> 17.5 U/ml	
HFV-seropositive*	8	4	Fisher's exact test $p < 0.05$
HFV-seronegative	11	0	

*at least one reproducible anti-HFV-env/gag serum antibody

**healthy reference < 14 U/ml, use as intestinal tumor marker > 37 U/ml

Table 3. Characteristics of patients with permanently detectable serum CA-19-9 (mean concentrations > 14 U/ml):

Patient /neopt. ⁵	CA-19-9	anti ¹ HFV	disease	duration	IgG ₃	visna ⁴	β_2 M
m71	149	env+, gag-	ALS ²	36 months	nma	-9%	nma
m63	79.5	env+, gag-	ALS	90 months	.37	-12.5%	1.3/3.7
f35	62.1	env+, gag-	ALS ¹	70 months	.29	-17%	nma
m69	26.5	env+, gag+	Parkins. ²	12 months	.65	-14.7%	7.0/19.4
f79	28.5	env+, gag+	Parkins. ³	12 years	.51	nma	nma
m33	19.6	env+/gag+	SMA ³ susp.	12 months	.31	-13%	1.3/4.8
m68	16.3	env-, gag-	ALS	74 months	.28	-5%	1.6/5.3
f75	15.5	env+, gag+	ALS	39 months	.44	-19%	1.9/8.8

ALS=amyotrophic lateral sclerosis, SMA=progressive spinal muscular atrophy, nma=no valid measurement available. 1) anti-human foamy retrovirus (HFV) antibody reactivity against recombinant envelope and/or capsid (gag) antigen (bold=very high titers/confirmed by immunoblot). 2) insulin-dependent diabetes mellitus for > 5 years. 3) no gastro-intestinal pathology confirmed. 4) competition with specific anti-visna retroviral antibodies (% reduction in binding to visna-maedi lentiviral antigen compared to healthy controls). 5) β_2 -microglobulin (mg/L) and neopterin (nmol/L) serum levels (underlined=elevated)

0.05); this reactivity correlated with anti-foamy retrovirus seropositivity ($p < 0.05$). In 25/28 ALS patients, we neither found any increase of neopterin as known from pulmonary or gastro-intestinal malignancies, nor decreased serum concentrations as seen in anxiety or schizophrenia (27). Neopterin and β_2 -microglobulin are markers of a cellular immune activation, regularly elevated in HIV, and increased in parts of human T-lymphotropic retrovirus (HTLV-1)-positive neurologic patients (16). Despite relatively progressed states of disease, including bulbar, hemiparetic, tetraparetic and moribund patients, neopterin and β_2 -microglobulin were not elevated in serum and singular samples of cerebro-spinal fluid; in neuroborreliosis, CSF neopterin increases in the absence of serum alterations. While activated macrophages have been found infiltrating spinal cord in ALS (14), basically normal β_2 -microglobulin and neopterin levels in amyotrophic lateral sclerosis argue against a persisting, generalized macrophage response in the disease.

The monoclonal antibody detecting carbohydrate antigen CA-19-9 recognizes a ganglioside containing glycosylated lacto-N-fucopentose-II (28). Both in HFV-seropositive ALS and HFV-antibody positive other neurological patients all negative for anti-GM₁ ganglioside antibodies, CA-19-9 concentrations seemed to be distributed similar to patients with non-malignant gastro-intestinal disorders (26). Maximal anti-HFV seroreactivity cosegregates with both anti-visna competition and elevated CA-19-9 serum ganglioside (Fig. 4). A cross-reactivity of unidentified macromolecules may contribute to anti-ganglioside reactivities in neurologic patients (29).

Anti HSRV-seronegativity (not anti-visna blocking activity) segregated with low IgG₃ serum concentrations. IgG₃ represents a predominant isotype in clearing intracerebral alphavirus encephalitic infection (32), and low IgG₃ concentrations are found in disimmune disorders and chronic fatigue syndrome (33). If HFV-seroreactivity does not characterize a disease variant, low circulating IgG₃ in HFV-seronegative ALS could result from specific or non-specific antibody sequestration. In ALS, clinical progression patterns (34), a "remarkably regional disease" (35), elevated circulating immune complexes (36, 8, 10, 37), non-inflammatory dermal alterations (10, 37, 38, 39), an inverse correlation between age and duration (10), and elevated lymphoma incidence (40, 11) partially remind of other retroviral diseases (41, 13). Nevertheless, we have neither observed any epidemiologic peculiarity of anti-retroviral seroreactive ALS patients, nor detected foamy viral genomic information in lymphocytes of HFV antibody-positive indi-

viduals up to now; there is, however, foamy-retrovirus immunoreactive antigenemia in ALS patients (unpublished). A regional, low-active retroviral expression, potentially expanding without completion of viral particles (42), would be compatible with both antiretroviral seroreactivities and a lack of evidence for generalized lymphoproliferation. In known retroviral central nervous system diseases with immune stimulation, increased concentrations are reported for interleukin-6 (43), soluble tumor necrosis factor receptor (44) or quinolinic acid (45). Though their production depends on macrophage stimulation and endogenous interferon-gamma production not observed in ALS, studies clarifying this issue are needed. In motor neuron disease in general, and ALS in particular, the immune system seems to be altered in rather peculiar ways (30), and younger patients or patients with multifocal conduction block, anti-ganglioside antibodies and immunoglobulin abnormalities may live longer than respective control patients (31). Whether retroviral serology, carbohydrate antigen, or immunoglobulin-G isotyping help identify diagnostic or prognostic subgroups remains to be elucidated.

Accompanied by diverse changes in the humoral immune response (46), the observed anti-retroviral seroreactivity could be due to a) an exogenous agent partially homologous to HFV and maedi-visna virus altering the host immune system (47), b) human endogenous retrovirus-like sequences (HERV) that may comprise 0.1-0.6% of the human genome (48) and mimic an anti-human foamy retroviral immune response, or c) a complex interaction of exogenous and/or endogenous retroviral sequences (49) with host genes or gene products. HFV indeed transactivates other retroviral agents (50). In experimental retroviral motor neuron disease, mice suffer a non-immunogenic retroviral injury to anterior horn neurons (12), and in natural rodent motor neuron disease, the translation products of endogenous murine leukemia retrovirus serve as surrogate receptor for another viral agent, lactate dehydrogenase-elevating virus (51). If transgenic mice defective for ciliary neuronotrophic factor (52) similar to mice expressing human foamy virus genes (4) die with progressive muscular atrophy, -though CNTF mRNA is preserved in ALS spinal cord-, a retroviral interference with cellular signaling may be responsible (53).

Acknowledgements

We thank Rolf M. Flügel, and Helmut Bannert, German Cancer Research Center D-69120 Heidel-

berg, for HFV serology, Norbert Sommer, Dept. Neurology, D-72076 Tübingen, for additional IgM GM-1 antibody determinations, S. Herzog and Rudolf Rott, Dept. Veterinary Virology, D-35392 Gießen, for anti-Borna disease virus screening, the RKU laboratory for coding assistance, and Hans Gelderblom, Berlin, and Kenneth Ricker, Würzburg for valuable discussion.

References

- Mahnke C, Löchelt M, Bannert H, Flügel RM Specific enzyme-linked immunosorbent assay for the detection of antibodies to the human spumavirus. *J Virol Methods* 1991; 29: 13-22.
- Bothe K, Aguzzi A, Lassmann H, Rethwilm A, Horak I Progressive encephalopathy and myopathy in transgenic mice expressing human foamy virus genes. *Science* 1991; 253: 555-559.
- Hoffmann PM, Pitts OM, Bilello JA, Cimino EF Retrovirus induced motor neuron degeneration. *Rev Neurol (Paris)* 1988; 144: 676-679.
- Aguzzi A, Bothe K, Wagner EF, Rethwilm A, Horak I Human foamy virus: an underestimated neuropathogen? *Brain Pathology* 1992; 2: 61-69.
- Westarp ME, Flügel RM, Clausen J, Föhring B, Kornhuber HH Evidence for human retroviral involvement in idiopathic non-familial amyotrophic lateral sclerosis. *J Neurol* 1992; 239: (Suppl. 2): 61.
- Westarp ME, Kornhuber HH, Rössler J, Flügel RM Human spuma retrovirus antibodies in patients with amyotrophic lateral sclerosis. *Neurol Psychiatr Brain Res* 1992; 1: 1-4.
- Flanagan M Isolation of a spumavirus from a sheep. *Austral Vet J* 1992; 69: 112-113.
- Apostolski S, Nikolic J, Bugarski-Prokopljec C, Miletic V, Pavlovic S, Filipovic S Serum and CSF immunological findings in ALS. *Acta Neurol Scand* 1991; 83: 96-98.
- Iwasaki Y, Ikeda K, Kinoshita M MRI lesions in motor neuron disease. *J Neurol* 1992; 239: 112-113.
- Ferro S, Salit IE; HIV infection in patients over 55 years of age. *J Acquir Immune Defic Syndr* 1992; 5(4): 348-353.
- Younger DS, Rowland LP, Latov N Lymphoma, motor neuron diseases, and amyotrophic lateral sclerosis. *Ann Neurol* 1991; 29: 78-86.
- Gardner MB Retroviral leukemia and lower motor neuron disease in wild mice: natural history, pathogenesis, and genetic resistance. In Rowland LP (ed): *Advances in Neurology* 1991; 56: 473-479.
- Engel WK Does a retrovirus cause amyotrophic lateral sclerosis? *Ann Neurol* 1991; 30: 431-433.
- Troost D, van dan Oord JJ, Vianney de Jong JM Immunohistochemical characterization of the inflammatory infiltrate in amyotrophic lateral sclerosis. *Neuropath Appl Neurobiol* 1990; 16: 401-410.
- Fuchs D, Weiss G, Reibnegger G, Wachter H The role of neopterin as a monitor of cellular immune activation in transplantation, inflammatory, infectious and malignant diseases. *Critical Reviews in Clinical Laboratory Sciences* 1992; 29: 307-341.
- Ali A, Rudge P, Dalgleish AG Neopterin concentrations in serum and cerebrospinal fluid in HTLV-I infected individuals. *J Neurol* 1992; 239: 270-272.
- Kuiper MA, Bergmans PLM, Teerlink T, Scheltens P, Wolters EC CSF biopterin and neopterin levels in Parkinson's disease patients. *Movement disorders* 1992; 7 (Suppl. 1): 74.
- Fuchs D, Albert J, Asjö B, Fenyö EM, Reibnegger G, Wachter H Association between serum neopterin concentrations and in vitro replicative capacity of HIV-1 isolates. *J Infect Dis* 1989; 160: 724-725.
- Mahnke C, Kashaiya P, Bannert H, Rössler J, Levin A, Blattner WA, Dietrich M, Luande J, Löchelt M, Friedman-Kien AE, Komaroff AL, Loh PC, Westarp ME, Flügel RM Human spuma retrovirus antibodies in sera from african patients. *Arch Virol* 1992; 123: 243-253.
- Bartholomä A, Muranyi W, Flügel RM Bacterial expression of the capsid antigen domain and identification of native gag proteins in spumavirus-infected cells. *Virus Res* 1992; 23: 27-38.
- Bechter K, Herzog S, Schüttler R Possible significance of Borna disease for humans. *Neurol Psychiatr Brain Res* 1992; 1: 23-29.
- Clausen J, Hoff-Jorgensen R, Rasmussen HB Antibody reactivity against animal retroviruses in multiple sclerosis. *Acta Neurol Scand* 1990; 81: 223-228.
- Have P, Hoff-Jorgensen R Demonstration of antibodies against bovine leukemia virus (BLV) by blocking ELISA. *Vet Microbiol* 1991; 27: 221-229.
- Fuchs D, Krämer A, Reibnegger G, Werner ER, Dierich MP, Goedert JJ, Wachter H Neopterin and β_2 -microglobulin as prognostic indices in human immunodeficiency virus type 1 infection. *Infection* 1991; 19: 98-102.
- Bartmann P, Grosch-Worner I, Wahn V, Belohradsky BH IgG; deficiency in children with human immunodeficiency virus infection. *Eur J Pediatr* 1991; 150: 234-237.
- Abbott Wiesbaden: Radioimmunoassay zur quantitativen Bestimmung des Carbohydrat-Antigens 19-9 (CA 19-9) in Humanserum oder -plasma, Wiesbaden 82-3578/R1, 1992.
- Sperner-Unterwieser B, Barnas C, Fleischhacker WW Is schizophrenia linked to alterations in cellular immunity? *Schizophrenia Res* 1989; 2: 417-421.
- Magnani J, Nilsson B, Brockhaus M, Zopf D, Steplewski Z, Koprowski H, Ginsburg V: The antigen of a tumor-specific monoclonal antibody is a ganglioside containing sialylated lacto-N-fucopentaose II, *Federation Proceedings* 1982; 41: 898.
- Weller M, Stevens A, Sommer N, Dichgans J, Kappler B, Wiethölter H: Ganglioside antibodies: a lack of diagnostic specificity and clinical utility? *J Neurol* 1992; 239: 455-459.
- Rowland LP Amyotrophic lateral sclerosis and autoimmunity. *N Engl J Med* 1992; 327: 1752-1753.
- Lange DJ, Trojaborg W, Latov N, Hays AP, Younger DS, Uncini A, Blake DM, Hirano M, Burns SM, Lovelace RE, Rowland LP: Multifocal motor neuropathy with conduction block: is it a distinct clinical entity? *Neurology* 1992; 42: 497-505.
- Parsons LM, Webb HE IgG subclass responses in brain

- and serum is Semliki Forest virus demyelinating encephalitis. *Neuropathology and Applied Neurobiol* 1992; 18: 351-359.
33. Peterson PK, Shepard J, Marcres M, Schenck C, Crosson J, Rechtman D, Lurie N A controlled trial of intravenous immunoglobulin G in chronic fatigue syndrome. *Am J Med* 1991; 89: 554-560.
 34. Küther G, Lipinski HG The dynamics of neuron degeneration in motor neuron disease: a theoretical approach. In Smith RA, *Handbook of Amyotrophic Lateral Sclerosis*, Dekker, New York 1992; 1992: 391-432.
 35. Munsat TL, Hollander D, Andres P, Finison L Clinical trials in ALS: measurement and natural history. *Adv Neurol* 1991; 6: 515-519.
 36. Oldstone MBA, Wilson CG, Perrin LH, Norris FH Evidence for immune complex formation in patients with amyotrophic lateral sclerosis. *Lancet* 1976; ii: 169-172.
 37. Westarp ME, Westphal KP, Kolde G, Wollinsky KH, Westarp MP, Kornhuber HH Dermal, serological and CSF changes in amyotrophic lateral sclerosis with and without intrathecal interferon beta treatment. *Int J Clin Pharmacol Ther Toxicol* 1992; 30: 81-93.
 38. Ono S, Mannen T, Toyokura Y Differential diagnosis between amyotrophic lateral sclerosis and spinal muscular atrophy by skin involvement. *J Neurol Sci* 1989; 91: 301-310.
 39. Kolde G, Ludolph AC Skin alterations in amyotrophic lateral sclerosis. *J Neurol Sci* 1990; 98 (Suppl): 75.
 40. Rowland LP Looking for the cause of amyotrophic lateral sclerosis. *New Engl J Med* 1984; 311: 979-981.
 41. Jolicoeur P, Rassart E, DesGroseillers L, Robitaille Y, Paquette Y, Key DG Retrovirus-induced motor neuron disease of mice: molecular basis of neurotropism and paralysis. *Adv Neurol* 1991; 56: 481-493.
 42. Sato H, Orenstein J, Dimitrov D, Martin M Cell-to-cell spread of HIV-1 occurs within minutes and may not involve the participation of virus particles. *Virology* 1992; 186: 712-724.
 43. Laurenzi MA, Siden A, Persson MA, Norkrans G, Hagberg L, Chiodi F: Cerebrospinal fluid interleukin-6 activity in HIV infection and inflammatory and noninflammatory diseases of the nervous system, *Clin Immunol Immunopathol* 1990; 57(2): 233-241.
 44. Cope AP, Aderka D, Doherty M, Engelmann H, Gibbons D, Jones AC, Brennan FM, Maini RN, Wallach D, Feldmann M Increased levels of soluble tumor necrosis factor receptors in the sera and synovial fluid of patients with rheumatic diseases, *Arthritis Rheum* 1992; 35: 1160-1169.
 45. Heyes MP, Mefford IN, Quearry BJ, Dedhia M, Lackner A: Increased ratio of quinolinic acid to kynurenic acid in cerebrospinal fluid of D retrovirus-infected rhesus macaques: relationship to clinical and viral status, *Ann Neurol* 1990; 27(6): 666-675.
 46. Westarp ME, Flügel RM, Jörgensen RH, Westarp MP, Kornhuber HH Serological parameters in amyotrophic lateral sclerosis (ALS). *J Neurol* 1992; 239 (Suppl. 3): 49.
 47. Leib-Mosch C, Bachmann M, Brack-Werner R, Werner T, Erle V, Hehlmann R: Expression and biological significance of human endogenous retroviral sequences, *Leukemia* 1992; 6 (Suppl. 3): 72-75.
 48. Acha-Orbea H Retroviral superantigens. *Chem Immunol* 1992; 55: 65-86.
 49. Pandey R, Gosh AK, Kumar DV, Bachman BA, Shibata D, Roy-Burman P Recombination between feline leukemia virus subgroup B or C and endogenous env elements alters the in vitro biological activities of the viruses. *J Virol* 1991; 65: 6495-6508.
 50. Lee AH, Lee KJ, Kim S, Sung YC Transactivation of human immunodeficiency virus type 1 long terminal repeat-directed gene expression by the human foamy virus bel₁ protein requires a specific DNA sequence. *J Virol* 1992; 66: 3236-3240.
 51. Contag CH, Harty JT, Plagemann PG Dual virus etiology of age-dependent poliomyelitis of mice. A potential model for human motor neuron diseases. *Microb Pathog* 1989; 6: 391-401.
 52. Sendtner M, Schmalbruch H, Stöckli KA, Carroll P, Kreutzberg GW, Thoenen H Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. *Nature* 1992; 358: 502-504.
 53. Westarp ME, Westphal KP, Clausen J, Rasmussen HB, Hoff-Jørgensen R, Föhring B, Kornhuber HH Retroviral interference with neurotrophic signaling in human motor neuron disease? *Clin Physiol Biochem*. 1993; 16: (in press).