# Passage of intravenous immunoglobulin and interaction with the CNS

Ulrich Wurster, Judith Haas

The success of intravenous immunoglobulin G (IVIg) therapy in immunhaematological disorders1 prompted us to use this treatment for neurological diseases of possible autoimmune origin. Several initial reports based on small numbers of patients showed encouraging results, and more recent controlled therapeutic trials confirmed the beneficial effect of IVIg in acute Guillain-Barré syndrome,2 chronic inflammatory demyelinating polyneuropathy,3 myasthenia gravis,4 intractable childhood epilepsy,5 and multiple sclerosis.6 In contrast with immunhaematological disorders, when the affected cells are virtually bathed in high concentrations of intravenously infused IgG, the access of IVIg to the nervous tissues is severely restricted by the blood-nerve, blood-brain and blood-CSF barriers. In this review the possible routes of entry of IVIg from the blood to the brain and cerebrospinal fluid will be considered and factors influencing this passage, as well as some possible mechanism of action, will be discussed.

## Intracerebral IgG concentration

The very effective seal produced by the tight junctions of the brain capillaries, should inhibit transsudation of IgG into the CNS. We could find no data on the concentration of IgG in brain interstitial fluid, but two related reports indicate the virtual absence of IgG in the brain parenchyma. When $^{99}$Tc-IgG was infused in patients with rheumatoid arthritis for the detection of inflamed joints by scintigraphy, no tracer was present inside the brain.[7] Likewise sections of brain cortex, cerebellum and brainstem of control guinea pigs showed no staining for IgG.[8] However, in the ependyma, the region of the hypothalamus and area postrema, which are part of the circumventricular organs that have a more permeable blood-brain-barrier, there was some diffuse IgG staining.[9] As no specific barriers exist between the extracellular fluid of the brain and the cerebrospinal fluid, similar concentrations of protein macromolecules may exist in the two compartments. Due to preferential drainage of brain interstitial fluid along perivenous spaces into the subarachnoid CSF and limited diffusion of ventricular CSF into the brain tissue to a few mm, the normal lumbar CSF IgG concentration of 20–40 mg/l should be considered as the approximate IgG content in the spinal cord and brain.

Nevertheless, the importance of sufficiently raised IgG levels in the CSF were clearly shown in a case of severe immunodeficiency with echovirus encephalitis, where only intraventricular application of IgG led to the elimination of the virus after massive doses of intravenous IgG had repeatedly failed to do so.[10] In HTLV-I associated myelopathy those patients who achieved higher IgG levels in their CSF after IVIg treatment (devoid of HTLV-I antibodies) showed a greater improvement.[11]

## Influence of serum concentration, size and charge of IgG

The concentration of a plasmaprotein in the CSF depends on its serum concentration, molecular size and charge. Although all these variables can be manipulated in favour of higher CSF IgG concentrations, there are considerable practical limitations. Maximal elevation of the serum IgG level is extremely expensive and carries the risk of plasma hyperviscosity. Also, raising the IgG serum concentration from the average value of 10 g/l to 30 g/l will only increase the CSF concentration from approximately 25 mg/l to 65 mg/l.

The use of F(ab')$_2$ (for example, Behring Gamma-Venin Ig 5S) fragments with about half the size of intact IgG would theoretically increase the transfer rate by a factor of two. However, such fragments have a very short intravascular half life of 12–36 hours compared with about 20 days for the intact IgG molecule. Moreover, functions associated with the Fc-part of the molecule would be lost altogether. The decisive influence of size became evident from a therapeutic attempt to treat patients with multiple sclerosis with a monoclonal mouse antibody against T12, a determinant on most post-thymic T-lymphocytes.[12] Whereas all T-12$^+$ cells were eliminated from the blood, such cells persisted in the CSF. Anti-T-12 levels in the CSF were barely detectable and attained less than 1/1000 of the serum concentration. This result was not surprising since the monoclonal antibody employed was of the IgM class. IgM has a molecular weight of 900 000 D, that is, more than five times larger than IgG with 160 000 D and will thus be almost completely excluded from the CSF.[13]

Cationisation would be another way to increase the transport of proteins across the choroid plexus and brain capillary walls. Raising the isoelectric point of albumin from 3·9 to 8·5 resulted in a tenfold higher transfer from blood to CSF.[14] Such a tremendous gain appears unlikely in the case of human IgG which already consists of rather basic molecules with isoelectric points from 5–9. Even so, when bovine $^3$H–IgG was brought to

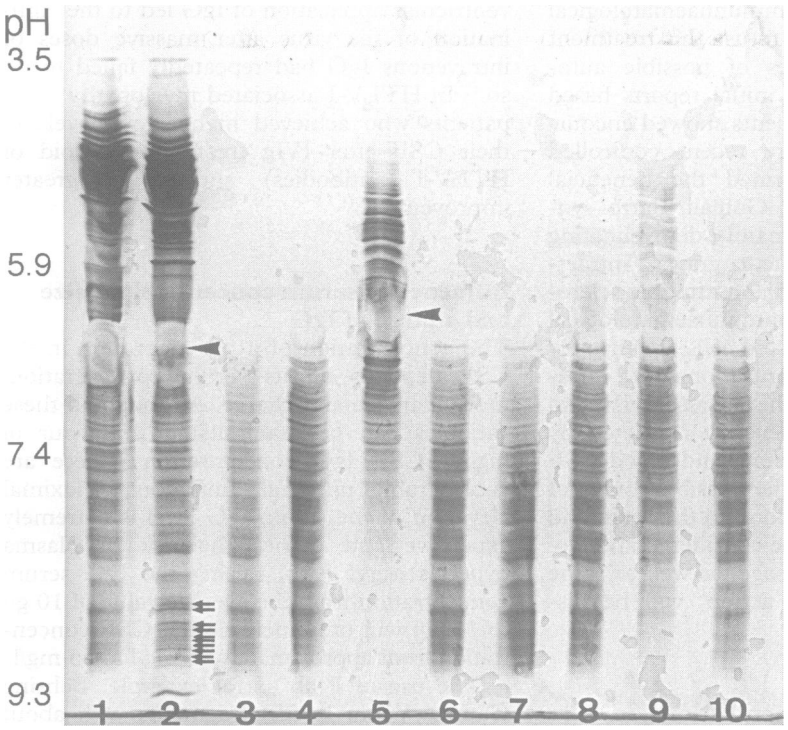
**CSF-Laboratory, Department of Neurology, Medical School, Hannover, Germany**
U Wurster

**Clinical Neurophysiology, Otto von Guericke Universität, Magdeburg, Germany**
J Haas

Correspondence to:
Dr U Wurster, CSF-Laboratory-7217, Department of Neurology, Medical School, D 30623 Hannover, Germany.

isoelectric points >10·7, brain uptake by absorptive mediated-endocytosis in Macaca monkeys was enhanced more than seven fold compared with native albumin.[15]

Because of the favourable effect of cationisation on the transfer of IgG, the introduction of negative charges appears to be less desirable. Isoelectric focusing of eight commercial IVIg preparations revealed two products (Intraglobulin F, Venimun) with anodical displacement of IgG (figure). Both substances have been chemically modified to reduce spontaneous complementary activity. Treatment of Intraglobulin F with β-propriolactone leads to the acylation of six lysine residues per IgG molecule[16] and S-sulphonation of Venimun preferably splits intra-chain disulphide bridges with the introduction of negatively charged sulphide ions. S-sulphonation is claimed to be reversible in vivo where the cleaved disulphide bonds would be restored by natural redox systems occurring in the blood.[17] The most anodic bands in the figure (lane 5 and 9) have been identified by Western blotting as IgA which is also contained in considerable amounts in these two preparations as can be seen in table 1.




*Isoelectric focusing pattern of serum, CSF and eight commercial preparations of intravenous immune globulin. Samples adjusted to 20 mg/l IgG were separated on polyacrylamide isoelectric focusing gels pH 3·5–9·5 (PAG–Plate, Pharmacia) and stained with silver. Lane 1: serum, lane 2: CSF of a patient with MS, lane 3: Alphaglobin, lane 4: Endobulin, lane 5: Intraglobin F, lane 6: Polyglobin N, lane 7: Purimmun, lane 8: Sandoglobin, lane 9: Venimmun, lane 10: Serapharm; (compare with table 1). Oligoclonal IgG bands in lane 2 have been marked with small arrows. Large arrow denotes the application point. Note the anodic displacement of IVIg in lanes 5 and 9.*

Apart from enhanced permeability there is yet another reason why it could be important that IgG preparations contain the full isoelectric spectrum of native IgG, especially the very basic fractions. More than 80% of the oligoclonal IgG produced by plasma cells in active MS plaques exhibit isoelectric points above pH 8·5 (figure). Given the reverse relationship between the charge of the antigens and that of the corresponding antibodies[18] highly alkaline oligoclonal IgG might be directed against unknown antigens of a more acidic character. IVIg preparations devoid of alkaline species might be unable to assist in the binding of such putative acidic antigens or would otherwise dilute the basic oligoclonal IgG. In the absence of any firm information on the role of oligoclonal IgG, the effects of adding an extraneous pool of human IgG inside the MS lesion must remain speculative.

## Specific uptake of IgG from blood and CSF

The barriers that include the epithelium of the choroid plexus, the endothelial cells of cerebral capillaries and the layer of cells lining

*Table 1 Characteristics of eight commercial preparations of immune globulin*

| *Brand name* | *Manufacturer* | *Form supplied* | *Manufacturing process* | *Additives* | *IgA (mg/l)* | *Il-6 (mg/l)* |
|---|---|---|---|---|---|---|
| Alphaglobin | Alpha Green Cross Langen | Liquid | PEG | 5% Sorbitol PEG 0·6% NaCl | <11 | 806 |
| Endobulin | Immuno Heidelberg | Lyophilised | Protease treatment PEG | 5% Glucose 0·5% PEG 4000 0·3% NaCl | 18 | 852 |
| Intraglobin F | Biotest Pharma Dreieich | Liquid | β-Propriolactone | 2·5% Glucose NaCl | 1370 | 69 |
| Polyglobin N | Cutter, Tropon Cologne | Liquid | pH 4·3 Diafiltration | 10% Maltose | 49 | 8186 |
| Purimmun | Armour Pharma Eschwege | Lyophilised | PEG Hydroxyethyl-starch | 2·5% Glucose 0·18% PEG 4000 Glycine, Citrate, Phosphate, Acetate | 20 | 1396 |
| Sandoglobin | Sandoz Nuremberg | Lyophilised | pH 4, traces of pepsin | 5% saccharose | 447 | 263 |
| Venimmun | Behring Marburg | Lyophilised | S-Sulfonation | 2·5% Glycine 0·85% NaCl | 3300 | 571 |
| Serapharm | Serapharm Münster | Lyophilised | PEG Bentonit | 2·5% Glucose 0·9% NaCl | 99 | 265 |

the arachnoidal membrane effectively block the penetration of plasma proteins, such as, IgG from the blood into the CNS under physiological conditions. However, there seems to exist some special mechanisms whereby these barriers may be partially circumvented. A tiny fraction (1%) of the brain capillaries in the region of the circumventricular organs are fenestrated allowing easier diffusion of protein macromolecules.

Although the presence of Fc-receptors on the basement membranes of the choroid plexus[19] would suggest the capability for active transport of IgG as in placental transfer, there is no evidence that such a mechanism is really taking place. As with any other plasma protein, IgG fits well into the function relating CSF/Serum quotients to molecular radius.[13] In contrast, CSF/serum quotients of transthyretin (prealbumin), cystatin C and $\beta_2$-microglobulin which are synthesised to a large degree locally by the choroid plexus[20] lie well above this line. The Fc-fragment of IgG did not enter the CSF significantly better than the F(ab) fragment which suggests that choroidal Fc receptors are not important for selective immunoglobulin entry.[14] Receptor mediated transport of IgG and IgA from plasma to CSF was also ruled out on the grounds that it was not isotype specific and that no secretory component was associated with IgA.[21] The Fc-receptors located on cells along the normal pathways of CSF outflow, including perivascular spaces, leptomeninges and arachnoid granulations may play a protective role in the clearance of IgG and antigen-antibody complexes in infections and immune-mediated disorders.[22]

A transport system for TNF-$\alpha$ was recently described as situated in the blood-brain-barrier of mice.[23] It is unclear at present whether a similar system could bring about a selective increase in transfer of IgG under certain (pathological) circumstances.[24] Conversely, motor neurons of the CNS with axons projecting outside the blood-brain-barrier where nerve endings are freely exposed to plasma proteins, seem capable of picking up IgG from *serum* by retrograde axonal transport in the rat.[25] Selective extraction of IgG from the *CSF* by dendrites of Purkinje cells has been demonstrated in the rat[26] and guinea pig.[8] In human necropsy studies normal IgG was detected in large amounts in the cytoplasm of Purkinje cells.[27] Antineuronal antibodies in patients with small lung cancer were found in dorsal root ganglia and Purkinje cells.[28] These observations offer a ready explanation for the successful early treatment of a case with paraneoplastic cerebellar degeneration by IVIg.[29] Since therapy was started within 14 days after onset of neurological symptoms the infused IgG stood a good chance of being taken up by the Purkinje cells and displacing the noxious antineuronal antibody, and at the same time preventing its further incorporation.

*Table 2 Effect of IVIg application on CSF IgG in a patient with chronic polyradiculitis*

| Day | IgG Ser (g/l) | IgG CSF (g/l) | Alb Ser/CSF | IgG Index | Rei/Fel[32] (mg/l) |
|---|---|---|---|---|---|
| −524 | 11·1 | 0·236 | 32·7 | 0·650 | −36·6 |
| 0* | 32·1 | 0·317 | 19·8 | 0·499 | −143·0 |
| 4 | 22·4 | 0·424 | 25·8 | 0·734 | −3·1 |
| 21* | 37·1 | 0·214 | 21·1 | 0·273 | −357·0 |
| 24 | 29·3 | 0·137 | 8·8 | 0·530 | −36·1 |

*30g IVIg (Serapharm). The upper limit for the CSF/serum quotient of albumin is 7·4, for the IgG index 0·7 and for Rei/Fel[32] 0 mg/l.

## IVIg entry and possible mechanisms of action under pathological conditions

The prospects of getting IgG into the CNS of a patient with an intact blood-brain/CSF barrier system seem to be limited. However, in many neurological diseases where treatment with IVIg has been attempted, some degree of barrier damage exists. The complete breakdown of the barriers in bacterial meningitis results in high local IgG concentrations, allowing the effective neutralisation of bacterial antigens and toxins.[30] One of the cardinal features of the Guillain-Barré syndrome is the high elevation of proteins in the lumbar CSF. The endothelial barriers of the dorsal root ganglia and ventral roots, which already leak more than under normal conditions, become severely damaged and permit the passage of plasmaproteins including IVIg.

The paramount influence of the barrier disturbance, expressed as the CSF/serum quotient for albumin, on the CSF IgG concentration of a patient with chronic polyradiculitis is evident from table 2. It takes about four days before equilibrium between the CSF and blood compartment is reached for IgG.[31] Calculation of intracerebral IgG synthesis on the days of IVIg administration therefore yields erroneously low IgG indices or excessively negative values with the hyperbolic formula of Reiber and Felgenhauer (see table).[32] As the state of the barrier approaches normality 24 days after the first application of IVIg, CSF IgG levels also fall despite sustained serum concentrations of 30 g/l.

Only about 20% of patients, mostly those with longstanding MS, exhibit a slight elevation in their albumin ratios, rarely exceeding values above 15. Yet the state of the blood-*CSF*-barrier is probably of minor importance for the access of IVIg to MS plaques. Of higher relevance is the breakdown of the blood-*brain*-barrier which is readily demonstrated by gadolinium enhanced MRI. Compared with normal white matter Gd-DTPA permeability was increased up to 30 fold in active MS lesions.[33] Not only the relatively small Gd-DTPA molecule with a molecular weight of 550 D, but also Gd-albumin and Gd-IgG accumulated in demyelinative lesions of rats with chronic EAE, an accepted animal model for MS. Interestingly, in three of six lesions, IgG seemed to be enriched over albumin, indicating selective transport or (un) specific binding of IgG.[34] The extravasation

of plasma proteins into the perivascular parenchyma of acute MS lesions was also revealed in a human post mortem histochemical study. The frequent colocation of IgG and C3d could signify the presence of immune complexes.[35] Since Fc-receptor expression is greatly enhanced on microglial cells in active MS lesions, antibody dependent cytotoxicity (ADCC) through the fixation of antimyelin antibodies might occur or immune complexes could be bound and phagocytosed by microglia causing liberation of cytokines and indirect damage by radicals released during oxidative burst.[36] If IVIg actually permeated into active MS lesion areas, blockage of the Fc-receptors on resident microglial cells or migrated macrophages could probably shut down the noxious events discussed above. Besides Fc-receptor blockade, the efficiency of IVIg in autoimmune disease has been attributed to idiotypic antibodies against autoantibodies.[1 37] Thus IVIg may exert its beneficial effect by the neutralisation of antimyelin antibodies. As active phases of MS are associated with increased titres of intrathecally produced antimyelin basic protein (MBP) antibodies, the removal of these autoantibodies directed against the anti-MBP was recently suggested as a possible therapy.[38]

Several conclusions emerge on the possible action of IVIg: 1) only IgG preparations with an intact Fc-part would be able to block Fc-receptors, for example, on microglia and macrophages; 2) immediate application of corticosteroids would appear to be counterproductive because of the known blood-brain-barrier restoring effect of such agents[39] which would hinder the influx of IVIg; 3) although the accessibility of IVIg to *chronic* MS lesions is hindered, external polyclonal IgG might promote CNS remyelination[40]; 4) it is not certain that it is necessary for IVIg to reach and interact with the CNS. In MS, numerous systemic aberrations have been described[41] and it is possible that IVIg helps to re-establish the immunological regulatory network by intercepting autoantibodies in the blood, induction of circulatory suppressor T-cells and long-term suppression of disease-related B-cell clones.

Finally, the source of IVIg in (costly) therapeutic trials is not necessarily an open choice, but depends on the company willing to sponsor the study. Table 1 illustrates the diversity of production processes and indicates the presence of noticeable amounts of IgA and IL-6 (measured by an ELISA) in certain preparations. However, the IL-6 content of the IgG product (Serapharm) employed in a trial of IVIg as an exacerbation therapy in MS was too low to explain the rise in IL-6 levels observed on day 3 and 10.[42] A similar increase in IL-6 concentrations had been noted after IVIg application in idiopathic thrombocytopenic purpura.[43] Soluble HLA molecules and their ligands CD 4 and CD 8 have also been detected in some IgG preparations.[44] At present it cannot be ruled out that impurities, additives or chemically altered IgG are responsible for some therapeutic as well as unwanted side effects of IVIg therapy.[45]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