Immunology of the Nervous System

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General preface to series

The impact of immunological thought on medical practice has been increasing at a steady rate now for nearly twenty years. There appear to be very few fields to which the immunologist cannot contribute. Initially the immunological approach was limited to assistance in diagnosis and in sera and vaccine production. New approaches in the field of therapy are not only in the use of vaccines, sera and immunosuppressive agents, but also in the more rational use of conventional therapeutic agents. Immunological knowledge is especially necessary in the field of tumour therapy, particularly in the balanced use of surgery and radiotherapy. Moreover, immunological knowledge in other fields has allowed us to understand more readily the mechanisms whereby a single aetiological agent can produce a wide range of different clinical manifestations. Different disease patterns occur depending on the nature of the immunological reaction causing tissue damage. A completely different symptom complex from reactions involving soluble immune complexes reacting with the complement cascade will be found in those involving the reaction of specifically sensitized lymphocytes with antigen as part of a cell-mediated or delayed hypersensitivity reaction.

As a massive amount of new scientific material accumulates in this field, the clinician is frequently left behind and perplexed. Each year a new scientific journal is published specializing in fields as diverse as immuno-genetics, immunochemistry or immunological techniques. We have journals emanating from continents as well as countries. The wealth of material is often bewildering. Simple textbooks of immunology are often too simple whereas review articles may be too complicated for the specialist physician or surgeon who wants a treatise on those aspects of the subject particularly relevant to his own field of interest. It is hoped that this series will fulfil some of these needs by giving comparatively short reviews that will lay emphasis on immunoligical subjects which should appeal to both clinicians and those working in clinical laboratories. The aim is to provide the busy clinician in a particular field of medicine with a short volume relevant to his practice written by a specialist. It should introduce the reader to the immunological approach to his subject and indicate how modern immunological thought might influence his day-to-day work in the wards or clinical laboratory.

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Preface

This monograph provides an outline of the immunology of the nervous system. It is primarily intended for clinicians, but will also be useful to immunologists and other research workers with an interest in neurological disease.

The immunopathology of the brain has a number of unusual features, which serve to distinguish lesions in the central nervous sytem from those in other tissues. These differences reflect the fact that the brain is relatively isolated from the general immune system. It lacks organized lymphoid tissue and is cut off from the circulation by a barrier which prevents the entry of antibody and immunocompetent cells. This means that the main defences of the normal brain lie outside the nervous sytem. The poliovirus, for example, must evade the defences in the gut, lymphoreticular organs and blood – and traverse the blood-brain barrier – before reaching the anterior horn cell. Nevertheless, the isolation of the CNS from the immune system is merely relative and may not be preserved during disease. With the onset of inflammation, the barrier is breached and lymhocytes as well as immunoglobulins enter the cerebrospinal fluid, whereupon the brain becomes an organ capable of an immune response and subject to a wide range of immunological reactions. These proceed against a background of blood-brain barrier phenomena which exert a modifying influence. The breakdown in the barrier is often transient and may be incomplete, with the result that the immune response in the CNS is delayed or restricted.

A second feature of the immunopathology is the unique way in which the specialized tissues of the brain – neurons and their supporting glia – respond to injury. A prime example is the process of demyelination, which can be produced by several immune mechanisms in the central and peripheral nervous sytem and has no counterpart in other tissues. In order to study these allergic demyelinating lesions, it has been necessary to supplement the usual immunological methods with techniques appropriated from neurobiology. A similar cross-fertilization between immunology and neurophysiology is apparent in the studies of myasthenia gravis, now recognized as an autoimmune disorder of neuromuscular transmission.

The opening chapters of the book deal with the blood-brain barrier and the immune response in the CNS. There follows an account of hypersensitivity reactions and autoimmunity, including experimental allergic encephalomyelitis, allergic neuritis and myasthenia gravis. Immunity in infectious disease and immune aspects of inflammatory disorders of unknown aetiology, including multiple sclerosis, are carefully considered. Subsequent chapters deal with the neurological manifestations of systemic immune disorders and the immune response to CNS tumours. The final section considers the application of immunological methods to the study of the structure and function of the brain.

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Immunity and the blood-brain barrier

For many years the brain was regarded as immunologically inert. Although this view is no longer tenable, it contains an important kernel of truth. The idea that immune reactions in the central nervous system (CNS) are restricted in some way arose early in the century from the study of the behaviour of transplantable tumours. It was observed that intracerebral grafts were notably more successful than those inoculated subcutaneously. They were usually from animals of the same species (allografts), but occasionally it was even found possible to cross the species barrier with successful xenografts (Murphy and Sturm, 1923). Viruses, too, appeared to flourish in the protected intracerebral environment. In 1930, Theiler showed that mice were highly susceptible to the yellow fever virus injected directly into the brain, and the intracerebral route of inoculation came to be used widely as a method of propagating viruses in the laboratory. The ease with which micro-organisms survived and multiplied in the brain suggested that it was immunologically unresponsive and effectively isolated from the immune processes of the body. The picture emerged of an organ which was unable to produce antibody itself, but was surrounded by a barrier preventing the entry of antibody or immunocompetent cells from outside. This view of immunity in the CNS was reinforced by some of the early work on tissue transplantation, which showed that the survival of skin allografts in the brain was prolonged, providing further confirmation of the belief that this organ was an 'immunologically privileged site.'

Over the years these views have had to be modified. We now know that grafts in the brain are rejected in much the same way as elsewhere (Scheinberg *et al.*, 1964); and that there may be an immune response to intracerebral tumours, and to viruses in the CNS. Nevertheless, it remains true that immunity in the CNS operates within certain constraints. The most important of these is that the normal brain contains virtually no immunocompetent cells. There are few lymphocytes, no lymph nodes and no conventional lymphatics, although one recent ultrastructural study has reported thin-walled structures in the Virchow-Robin space, which may be lymphatics of a kind (Prineas, 1979). The absence of organized lymphoid tissue reflects the fact that the brain is not normally exposed to significant levels of antigenic stimulation. It is an inaccessible organ, shielded from attack by invading organisms, and relatively impermeable to antigens reaching it from the bloodstream. Lacking both immunocompetent cells and antigenic stimulation, the normal brain

does not synthesize antibody, and the small amount of immunoglobulin in the CSF is derived from the plasma. It is hardly surprising that immunological reactions in neural tissue are often muted and frequently delayed. It has gradually become apparent that the immunological privilege of the brain is not absolute – but relative – and that its limited capacity to react depends upon the integrity of the barriers separating it from the circulating blood. In disease, these barriers break down and the inflammatory process allows lymphocytes and antibody to enter the nervous system. At this point the brain becomes an organ capable of generating an immune response.

The CNS, therefore, displays a wide spectrum of immunological behaviour, ranging from relative unresponsiveness at one end, to the most florid manifestations of hypersensitivity an immunity at the other. The persistence of many viral infections and the tardy rejection of some allografts testify the degree to which the brain is isolated from the general immune system. On the other hand, diseases such as multiple sclerosis (MS) and subacute sclerosing panencephalitis (SSPE) are associated with active antibody synthesis in the CNS; while experimental allergic encephalomyelitis (EAE) in animals provides a striking illustration of the fact that hypersensitivity can produce devastating lesions in the brain. In order to appreciate the unusual character of the immune response in the nervous system, it is necessary to start by considering the mechanisms which regulate and restrict the entry of antigens, antibody and immunocompetent cells into the brain.

The blood-brain barrier

The cells of the brain function in an environment which differs from that of other organs. This unique milieu is maintained by a series of functional and anatomical barriers regulating the entry of fluid, electrolytes, small molecules and protein from the blood into the extravascular space (Gardner, 1972; Rapoport, 1976; Bradbury, 1979). The neural tissues are therefore surrounded and permeated by a fluid which differs markedly from the intercellular fluid elsewhere. From an immunological point of view, the most important difference is the low concentration of protein and, in particular, of immunoglobulin. In most tissues the interstitial fluid is derived from the blood by a process of filtration and vesicular transport through the walls of the capillaries. It bathes the parenchymal and connective tissue cells and after entering the lymphatics, drains to the local and regional lymph nodes. The transudate, which leaves the arterial end of the capillary, contains only 3000 mg/l of protein, but as a result of the reabsorption of water at the venous end, the protein concentration in the interstitial fluid may reach 18 000 mg/1. This fluid resembles plasma and contains most of the major plasma proteins, including immunoglobulin. It drains into the lymphatic channels, where the protein concentration is still further increased. The amount of protein in the prenodal lymph varies in different tissues, but in general it is about 40 per cent of that in plasma (Yoffey and Courtice, 1970). Connective tissue is therefore bathed in a fluid which has a protein concentration of 28 000 mg/l compared with 70 000 mg/l in plasma.

The concentration of IgG, the major immunoglobulin component, is

proportionately reduced, although it is important to bear in mind that the interstitial fluid volume is large and half of all the IgG in the body is extravascular. The comparison with the brain is striking. The major extravascular compartment in this organ is the cerebrospinal fluid (CSF), where the protein concentration is 200-400 mg/l. This is about 0.4 per cent of the serum level, and means that the CSF protein concentration is reduced to 1/250th of that in the serum (Heremans, 1966).

The exclusion of serum protein from the nervous system was demonstrated visually by injecting dyes, such as trypan blue or Evans' blue, into experimental animals. The dyes bound to protein and quickly equilibrated in the circulation, and some of the labelled protein diffused out and coloured the tissues. Brain and cord remained unaffected, although they could be stained by intracerebral injection. From these observations arose the concept of a barrier interposed between the circulating blood and the CNS, regulating the entry of substances into the brain. This selectivity was supposed to apply to drugs and other injected material, as well as to normal constituents of plasma and metabolites. The blood-brain barrier was originally seen as a single exclusionary interface, but the situation is more complex. There is no single barrier but a *series of regulatory interfaces*, which determine the rate at which substances pass into the brain (Rapoport, 1976). A variety of processes is involved, including diffusion, active transport and pinocytosis. The rate of passage is regulated by the nature of the cerebral capillary and the metabolic activity of the glial cells. An additional factor is the closely packed structure of neural tissue with its restricted intercellular space, resulting in many substances passing through cells, rather than diffusing in between. In the case of protein, however, the original concept of an exclusionary barrier is still valid. Its movement from the plasma into the CNS is restricted at a complex interface made up of three surfaces – the epithelium of the choroid plexus, the endothelial cells of the cerebral capillaries and the layer of cells lining the arachnoid membrane. It is at these surfaces that plasma is filtered to form the extravascular fluid.

In the brain, the extracellular fluid is contained in three compartments, blood, interstitial fluid and CSF. The last two together constitute the extravascular space. Protein in this space is derived from plasma by diffusion, although some minor components originate within the brain itself.

The cerebrospinal fluid

The brain and cord are suspended in the cerebrospinal fluid, which also fills the ventricles. The fluid is derived from the plasma, 70 per cent being produced by the choroid plexus. The remaining 30 per cent is derived from the capillary bed of the brain and meninges (18 per cent) or metabolic water production (12 per cent). It passes out of the ventricular system via the foramina of the fourth ventricle and enters the subarachnoid space, where it is reabsorbed into the blood by the arachnoid granulations of the superior sagittal sinus. The CSF has a volume of about 140 ml and is secreted at the rate of 0.35 ml/min., which means that the entire volume is replaced three to four times a day (Davson, 1967; Bradbury, 1979). Fluid secreted by the plexus first filters through the walls of the choroidal capillaries, which have a loose fenestrated appearance on electron microscopy. The composition of the filtrate is similar to that of interstitial fluid elsewhere, but

protein is prevented from entering the CSF by the surface lining of cuboidal cells, forming the choroidal epithelium. These cells are connected by tight junctions, which form a continuous sheet of epithelium which restricts the passage of protein and is responsible for its low concentration in the CSF. Fewer than 1 protein molecule in 200 succeeds in crossing this barrier (Fig. 1.1).

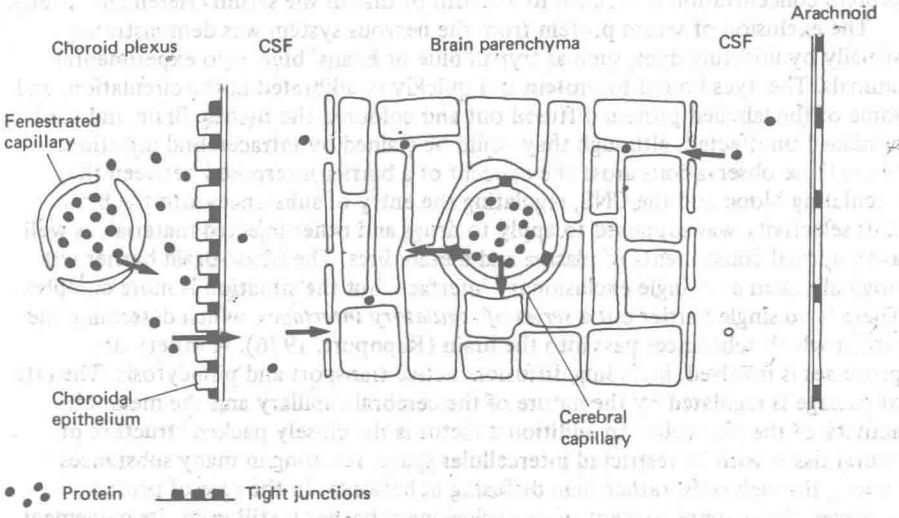

Fig. 1.1 The blood-brain barrier to protein.

The site at which proteins are held up has been demonstrated by injecting peroxidase intravenously. This marker has a molecular weight of 17 000 and can be traced in the tissue by electron microscopy. It passes through the walls of the capillaries without difficulty, but is arrested at the tight junctions connecting the apical regions of the epithelial cells, and fails to enter the CSF (Brightman and Reese, 1970).

The intercellular space

The CSF bathes the surface of the brain and cord, and the ependymal lining of the ventricular system, but these surfaces do not constitute a barrier to the diffusion of protein. Peroxidase injected into the subarachnoid space or ventricles penetrates the narrow clefts between the brain cells without difficulty and enters the parenchyma. The CSF and the interstitial fluid are, therefore, in communication and may be regarded as a single compartment – the extravascular fluid space of the brain.

The nervous system is derived from ectoderm, and its cells, like those of the epidermal layer of the skin, are closely packed. The interstitial space is small, although its exact dimensions are still disputed. On electron microscopy, the clefts between the cells are about 2 nm wide, varying with the method of fixation. Physiological estimates of the space are more generous, varying from 5 per cent to

20 per cent of the total brain volume (Kuffler and Nicholls, 1977). The interstitial fluid is mainly derived from the capillaries of the brain parenchyma, which are lined by endothelial cells, joined by tight junctions producing a filtrate low in protein, resembling the CSF. The passage of substances from the blood is further impeded by the astrocyte foot processes which are closely applied to the capillary wall and cover 70 per cent of its surface. Protein finding its way into the interstitital space will diffuse out into the parenchyma, although the rate of diffusion will be limited by the narrowness of the intercellular clefts. The movement of large molecules, such as immunoglobulin, will be affected more than that of smaller ones, such as albumin.

The epithelium of the choroid plexus and the endothelium of the cerebral capillaries are two barriers to the entry of protein. A third is the layer of arachnoidal cells, which prevents protein diffusing into the CSF from the outer layers of the arachnoid. These three surfaces mark the functional boundaries of the extravascular fluid space (Fig. 1.1).

However, the blood-brain barrier is incomplete. There are certain discrete areas where protein molecules may enter more easily. They include the posterior pituitary, pineal, tuber cinereum and a small area in the floor of the fourth ventricle, the area postrema. These regions of the brain stain blue when the dye Evans blue is injected intravenously. Diffusion into these areas may contribute protein to the CSF and play a role in immunopathology by allowing the passage of antibody into the CNS.

In this account of the formation of CSF we have assumed that the plasma proteins enter the fluid compartment of the brain by diffusion. This is largely true, but exceptionally other processes may be involved. In the case of at least two plasma constituents, pre-albumin and transferrin, the concentration is higher than that predicted on the basis of diffusion alone, and it is possible that they may be secreted by the lining cells of the choroid plexus. This would be an energy-requiring process – a form of active transport. Some protein may also pass across the endothelial cells of the cerebral capillaries by pinocytosis. However, vesicular transport of this kind is considered unimportant in the CNS, and most of the serum proteins enter the brain by diffusing between cells, rather than passing through them.

Although the cerebrospinal fluid occupies a single intercommunicating space, there are differences in the composition of the fluid at different levels. The total protein concentration in the lumbar region is higher than that in the ventricle (Heremans, 1966). The difference is due to diffusion from the subarachnoid and meningeal vessels, which makes a greater contribution to protein in the lumbar than the ventricular CSF. A similar difference exists between lumbar, cisternal and ventricular immunoglobulin levels and must be taken into account when considering antibody levels in the nervous system.

Proteins in the cerebrospinal fluid

The CSF proteins can be separated by electrophoresis on cellulose acetate, or in agarose or starch gel; if allowance is made for the difference in concentrations, the electrophoretic pattern is similar to that of serum (Lowenthal, 1965). About half

of the protein is albumin, which runs as a dense band towards the anode, followed by the α_1-, α_2- and β-globulins, and a faintly staining diffuse γ-component. There are two small additional fractions – the first running ahead of the albumin and a second in the slow β-region. The fast component is due to a protein similar to the pre-albumin of serum. It has a slightly smaller molecular weight than albumin and the reason for its relative abundance in the CSF is unknown. The second was originally referred to as the τ-fraction, but was subsequently resolved into several distinct subfractions including transferrin, β_2-microglobulin and a protein referred to as β-trace. A discrete minor component and β-trace may also be observed trailing at the cathodal end of the γ-fraction (Heremans, 1966, Laterre, 1973).

The immunoglobulin runs as a faintly staining diffuse band in the γ-region, extending into the β and α_2. Immunoglobulin G and frequently IgA can be identified by immunoelectrophoresis, producing arcs of precipitation in the same positions as they do in serum.

The development of cellulose acetate as a support medium for electrophoresis greatly simplified the technique and allowed the introduction of CSF electrophoresis as a routine procedure. However, the method lacks resolving power in the γ-region and, for the analysis of immunoglobulin, agarose is the method of choice (Johnson *et al.*, 1977). One major disadvantage is that the CSF has to be concentrated 50-200 times before use, which may lead to denaturation, precipitation or aggregation of protein, and produce art facts in the form of discrete bands. This difficulty may be overcome by using polyacrylamide gel, which only requires a small amount of unconcentrated CSF (Thompson *et al.*, 1979). It has high resolving power and as many as twenty bands may be identified in normal fluid (Fig. 1.2). An even greater resolution may be obtained with the recently developed technique of isoelectric focusing (Laurenzi and Link, 1978a and 1978b; Olsson and Nilsson, 1979; Hosein and Johnson, 1981). In this method, proteins migrate under an applied voltage to their isoelectric points in a pH gradient established in polyacrylamide (Fig. 1.3). This produces a concentration effect giving rise to very sharp and discrete protein bands. Polyacrylamide and isoelectric focusing are widely used in research, although cellulose acetate and agarose retain their place in the routine laboratory.

After separating the proteins, the individual components can be identified by immunoelectrophoresis or immunofixation. In the former, a specific antiserum is allowed to diffuse into the medium at right angles to the original electrophoretic separation, and the arc of precipitation which forms with the antigen defines its position in the gel.

Immunofixation allows individual proteins to be identified with even greater precision. After electrophoresis, specific antiserum is applied to the gel or paper surface, reacting with the antigen and fixing it. The gel is then washed with buffer to remove all soluble protein and the precipitated antibody-antigen complex shows up as a sharply defined band when stained with a protein dye such as amido black (Fig. 1.3). By comparing its position with the original electrophoretic pattern, the precise location of the component can be determined (Laurenzi and Link, 1978b; Stibler, 1979). Increased sensitivity can be obtained using radiolabelled antibody and visualizing the line of precipitation by autoradiography.

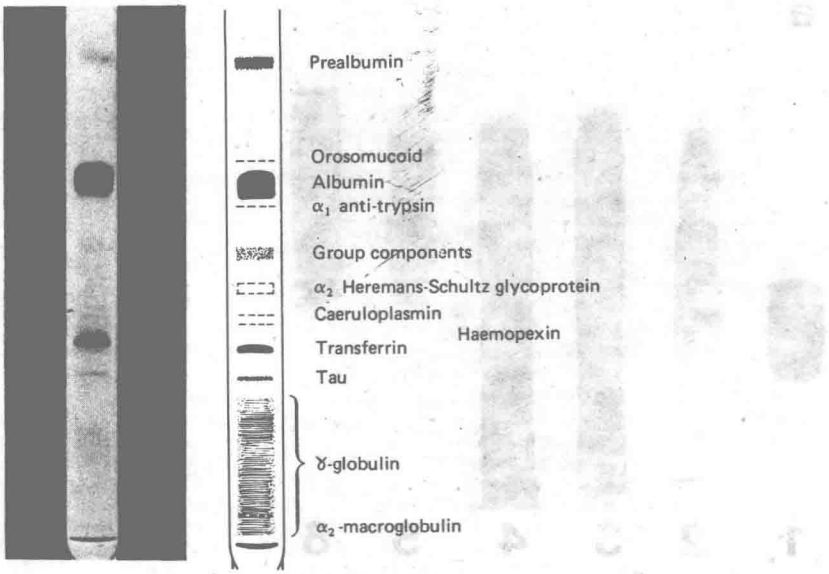

Fig. 1.2 Electrophoresis of normal CSF in polyacrylamide gel. (Courtesy of Dr E. Thompson.)

This is usually done in two stages. The gel is first traced with specific antiserum. If, for example, one wishes to identify the position of IgM in the electrophoretic strip, the gel is first treated with, say, antibody to human rabbit anti-IgM and subsequently with radiolabelled anti-rabbit immunoglobulin. It is then placed in contact with a strip of photographic film in a dark container and the film is subsequently developed to reveal the position of the band of radioactivity. Many proteins of immunological importance have been identified in CSF, including the immunoglobulins (IgG, IgA and traces of IgM), β_2-microglobulin and the complement components C3 and C4.

Immunoglobulin

Since proteins enter the CNS by diffusion, the rate at which they cross the blood-brain barrier is related to their plasma concentration. High concentrations increase the diffusion gradient and raise the level in the CSF. For this reason albumin, which is the major serum protein, is present in greatest concentration in the spinal fluid. There is less immunoglobulin in the serum and the level in CSF is correspondingly reduced. Diffusion is also inversely related to molecular size, and small molecules cross the barrier more easily than large ones. Albumin, with a molecular weight of 67 000, diffuses more rapidly than IgG (190 000) and proportionately more of it enters the CSF. This is reflected in their CSF : serum ratios, which are 1 : 200 for albumin and 1 : 250 – 1 : 500 for IgG. The even larger molecule of IgM circulates in the plasma as a pentamer with a molecular weight of 900 000. This large size hampers diffusion into the extravascular space and it is virtually excluded from the CSF.