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Reviews

Neuronal control of brain microvessel function

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Summary. Cerebral capillary endothelium forms a barrier limiting and controlling the movement of ions and solutes between blood and brain. Recent anatomical, physiological and biochemical studies have suggested the possibility that capillary function may be directly controlled by neuronal structures. Alterations in neuronal systems involved in the regulation of microcirculation may account for microvascular dysfunctions which occur in different pathologic conditions.
Key words. Brain; capillary function; regulation; microvasculature, innervation; cerebral blood flow; vascular permeability.

Introduction

The microvasculature of the brain differs from that of other tissues in certain aspects: cerebral blood flow and oxygen consumption are higher than in other organs and the movement of substances between blood and brain parenchyma is regulated by particular mechanisms.

Blood vessels supplying the brain may be classified into four types: inflow tract arteries, arteries, intraparenchymal arterioles and capillaries. Inflow tract arteries, pial arteries and arterioles containing muscle structures take part in the regulation of cerebral blood flow.

It is known that larger vessels and arterioles receive monoaminergic, cholinergic and peptidergic innervation, as reported by various studies[13,19–23,41,66,83]. In this review we describe recent evidence supporting the existence of a neuronal control of brain microvasculature. Cerebral capillaries constitute part of the blood-brain barrier which limits the transendothelial transport of molecules and ions from the blood to the brain parenchyma. Along this line, the knowledge of the mechanisms regulating microvessel function may have potential clinical implications in some physiological and pathological states such as aging, cerebrovascular disorders and hypertension.

Anatomical studies

Various ultrastructural studies have demonstrated that pial and larger intraparenchymal blood vessels are closely associated with a *catecholamine-containing nerve plexus* which originates from the superior cervical ganglion (for review see Edvinsson[20]). More recent evidence suggests that, in addition to the sympathetic chain, there may be a central source of adrenergic innervation of brain microvasculature. In particular, dopamine beta-hydroxylase immunohistochemistry and fluorometrical histochemistry have shown that intraparenchymal small vessels and capillaries are closely associated with adrenergic terminals which persist after superior cervical ganglion sympathectomy[24,25,38,39]; this finding suggests that fibers may originate from brain stem nuclei, such as the locus coeruleus[25]. Electron microscopical studies have shown the presence in the hypothalamus and cerebral cortex of adrenergic varicosities containing large and small core vesicles in close contact with the basement membrane of capillary endothelial cells[103,114]. Although there is no proof that varicosities apposed to the basal lamina constitute synaptic specialization, this finding provides additional evidence of an intrinsic adrenergic innervation of cerebral microvasculature. This hypothesis has stimulated a number of biochemical and physiological studies on the adrenergic mechanisms regulating the blood-brain barrier function.

Cholinoesterase histochemistry and electron microscopical studies have demonstrated the *cholinergic innervation* of pial vessels[22,26]. More recent observations have revealed the existence of non-aminergic nerve terminals containing non-core vesicles in direct contact with the basal lamina of intraparenchymal small vessels[114]; this kind of axon varicosity was suggested to be cholinergic. Furthermore, using other experimental techniques, acetylcholinoesterase-staining fibers have been demonstrated to approach cerebral microvasculature[3,48,55]. The

origin of the cholinergic innervation is still controversial; however, denervation studies have shown that pial vessels receive parasympathetic fibers running through the facial nerve to the geniculate ganglion[68,104], while intraparenchymal small vessels receive cholinergic innervation originating from the brain stem[3,55].

A serotoninergic innervation of cerebral arterioles has been demonstrated by means of several methodological approaches, such as autoradiography[13], fluorescence histochemistry[18,49], immunohistochemistry[37] and biochemical methods[27]. Biochemical data also indicate the existence of serotoninergic projections to cerebral microvasculature and capillaries[102]. Serotoninergic pathways originate from the raphe nuclei located in the brain stem[102].

Immunohistochemical studies have revealed a *peptidergic innervation* (Vasoactive Intestinal Peptide or VIP and Substance P or SP) of larger cerebral vessels[13,23,66]; occasionally, VIP-immunoreactive nerves have also been observed along intracerebral vascular branches[66]. Both VIP- and SP-fibers have been shown to dilate cerebral vessels and to increase cerebral blood flow[66,30,77,117], suggesting a role in the regulation of brain circulation. No evidence is at present available concerning the existence of VIP- and SP-containing fibers supplying the capillary bed.

Recent works by Hendry and coworkers have demonstrated that cholecystokinin (CCK) immunoreactive neurons in the cerebral cortex make close contact with small brain vessels[43]. This finding raises the possibility of a role for CCK in the regulation of cerebrovascular function.

Physiological studies

The identification of neurotransmitter pathways supplying cerebral microvasculature has suggested the existence of a neuronal regulation of the blood-brain barrier. Most of the physiological studies deal with the influence of the central adrenergic system on cerebral microvascular function; in particular direct evidence is provided by investigations on the role of the locus coeruleus, the cellular origin of adrenergic fibers innervating brain microvessels, in the regulation of cerebral blood flow and capillary permeability.

a) *Cerebral blood flow*

Small arteries and arterioles are the elements of cerebral vascular bed that primarily control, or change, the blood flow in the brain. The influence of various neuronal systems (adrenergic, serotoninergic, cholinergic, peptidergic) on these blood vessels has been extensively investigated and reviewed[13,19,20,23,41,66]. As regards intraparenchymal small vessels and microvasculature, current evidence suggests that adrenergic neurons in the locus coeruleus play a role in the control of microvascular function. In fact, lesions of the central adrenergic system result in increase in resting cerebral blood flow and in a significant loss of vascular response to hypercapnia; conversely, the stimulation of the locus coeruleus tends to decrease cerebral blood flow[99,100]. The involvement of brain capillaries (5–10 μm diam.) in the control of local blood flow is still controversial. Supporting this hypothesis, recent studies have shown the presence of the contractile proteins, actin and myosin, also in capillary endothelial cells[67,88,89]. However, the possibility that contractile proteins may have a role in the mechanisms of pinocytotic capillary transport rather than in the regulation of local blood flow should be taken into account.

b) *Permeability*

It is well known that the diffusion of many water-soluble substances, such as ions, proteins and polar organic compounds, into the brain parenchyma is limited. The restricted movement between the blood and the brain has been attributed to the peculiar characteristics of cerebral microvessels compared with those of other organs. In fact, ultrastructural studies have shown that brain capillary endothelial cells are closed by tight junctions which represent a sort of anatomical barrier limiting the diffusion of solutes into the brain. Furthermore, in contrast to peripheral microvessels, cerebral capillaries show a low rate of transendothelial transport by pinocytotic vesicles[17,101,110]

In addition to the concept of 'anatomical' barrier, the existence of a metabolic blood-brain barrier for certain substances is indicated by the presence in endothelial cell plasma membranes of specific enzymes (e.g. DOPA decarboxylase, monoamine oxidase and catechol O-methyl transferase) which metabolize and control the diffusion of monoamines and amine precursors to the brain[7].

Brain vasculature is dynamically regulated to respond to various stimuli. Metabolic changes, circulating hormones and neurogenic factors may cooperate in the control of cerebral capillary permeability. Physiological evidence supporting a neuronal regulation of the blood-brain barrier has come from a number of studies. In particular, it has been suggested that the passage of relatively diffusible substances, such as ethanol and water, into the brain may be influenced by the central adrenergic system. Raichle et al. have demonstrated that the chemical and electrical stimulation of the locus coeruleus in bilaterally sympathectomized monkeys produces a marked and rapid increase in the cerebral extraction of water[99]. The fact that under such conditions cerebral blood flow is reduced may indicate that the increased water extraction actually results from an increase in capillary permeability. Both the effects on cerebral blood flow and water permeability are blocked by the adrenergic antagonist phentolamine.

Further investigations have demonstrated that tricyclic antidepressants, which inhibit catecholamine re-uptake and consequently increase the concentration of the neurotransmitter at synaptic level, alter the blood-brain barrier function by increasing the penetration of water and ethanol into the brain, independently of cerebral blood flow[97]. The same authors have also observed that lithium administration and electroconvulsive shock, which attenuate the activity of central adrenergic neurons, reduce the increase in water permeability induced by the elevation of blood CO_2 tension[97]. These findings may provide evidence on the possibility of an adrenergic regulatory mechanism of cerebral microvasculature.

A carrier mediated active transport system into the brain has been described for ions and solutes, such as potassium ions and amino acids[8,16,34]. Recently, Betz et al.[9] proposed a polar model for capillary endothelium, based on a different and selective distribution of transport proteins between the opposite surfaces of the cell. For example, γ-glutamyltranspeptidase, which is involved in the transport of large neutral amino acids across the blood-brain barrier, is present in both the antiluminal and luminal membranes, whereas Na^+-K^+-ATPase, involved in the transport of Na^+ and K^+ ions, is only present on the antiluminal surface of endothelial cells. The existence of cellular polarity would permit vectorial transport against concentration gradients across the capillary wall. Although no experimental evidence is available, the possibility that neurotransmitter pathways to brain microvasculature may control the transport of certain molecules, such as amino acids into the brain should be taken into account.

Neurochemical studies: receptors located on microvessels

Several techniques have been recently developed to obtain purified metabolically active cerebral microvessels[10,35,54]. Under the electron microscope, the microvascular enriched fraction isolated from the brain appears to be mainly composed of endothelial cells and pericytes surrounded by the basement membrane. Occasionally, a few astrocyte endfeet were visible outside the basal lamina[116]. Precapillaries and arterioles may also be found with smooth muscle cells still attached. Neuronal cells are absent and a very small percentage of free nuclei is observed. γ-glutamyl transpeptidase (γ-GTP), an enzyme localized in the capillary wall, is enriched by about 20-fold in microvascular fractions compared with the brain homogenate[35]. Thus, the measurement of γ-GTP may allow the biochemical assessement of the purity of the preparation. The integrity of metabolic pathways (glycolysis, fatty acid oxydation, etc.) and transport systems was shown to be preserved in isolated brain vessels[10]. Thus, although a certain degree of contamination from glial elements is present in all the preparations, the development of techniques for the purification of intact brain microvessels has permitted investigation on the nature of the capillary in vitro. Receptor binding radioassay and measurement of the cyclic nucleotide-generating system interacting with neurotransmitters have directly shown the existence of receptor sites in brain microvessels which may mediate the action of circulating as well as neurogenically released factors.

1) *Norepinephrine*

In cerebral microvessel preparations, the existence of norepinephrine[65], its synthesizing and catabolizing enzymes[65], beta-adrenergic sensitive adenylate cyclase[44,80,90] and β-adrenergic receptor sites[57-61,95] has been well documented.

Epinephrine is more potent than norepinephrine in stimulating beta-adrenergic-sensitive adenylate cyclase. The enzyme is preferentially but not completely blocked by $beta_2$-antagonists, indicating that most adenylate cyclase-associated adrenergic receptors in microvessels are $beta_2$-type[81,91]. Iodohydroxybenzylpindolol (IHYP) binding to beta-receptors on brain microvessels is inhibited by isoproterenol, epinephrine and norepinephrine. Modified Scatchard analysis of the inhibitory effects of practolol, metoprolol and zinterol on IHYP binding has provided direct evidence for the existence of two populations of β-adrenergic receptors in brain capillaries[59]. The majority of them (about 80%) is of beta 2-type.

Isoproterenol, but not terbutaline ($beta_2$-agonist), increases blood flow in certain brain regions. This effect is selectively blocked by $beta_1$-antagonist practolol[69], indicating that $beta_2$-adrenergic receptors in cerebral microvessels do not have an important role in the regulation of cerebral blood flow. It is possible that $beta_2$-receptors in cerebral microvessels do not just play a role in the control of blood flow; in particular, they may be involved in the regulation of capillary permeability, as described above.

Receptor sites for alpha-adrenergic ligands, ^{3}H-WB 4101[95], ^{3}H-prazosin and ^{3}H-para-aminoclonidine[60,61], have been reported in brain microvessels; these receptors may also have a role in the regulatory mechanisms of microvascular function[61,97-99].

2) *Acetylcholine*

Recent reports indicate the existence in brain capillaries of a high affinity muscarine type acetylcholine receptor for ^{3}H-quinuclydil benzilate associated with high choline acetyltransferase activity[31]. It is known that the administration of acetylcholine increases cerebral blood flow by reducing brain vascular resistance and that these effects are attenuated by atropine[2,15]. The presence of muscarine receptors and the anatomical identification of cholinergic nerve terminals associated with small intraparenchymal vessels raise the possibility that some functions of the capillaries, such as the regulation of cerebral blood flow and transendothelial transport, may be in part influenced by cholinergic pathways.

3) *Dopamine*

Ultrastructural studies in the rat have revealed that dopaminergic neuron dendrides are closely associated with the capillary endothelial cells[14], while biochemical observations suggest the existence in microvascular enriched fractions of dopamine-sensitive adenylate cyclase[4]. However, dopaminergic receptors in cerebral capillaries have not been detected by our and other laboratories.

4) *Serotonin*

In vivo and in vitro studies have shown that serotonin is a potent vasoactive substance[84,107]. Its effects on blood flow are complex and dependent on the vascular district[71]. As regards cerebral microcirculation, it has been postulated that serotonin may, in part, influence vascular reactivity via brainstem mechanisms. The suggestion of serotonin neuronal projection from raphe nuclei to small cerebral vessels comes from recent biological data. In fact, it has been demonstrated that the electrolytic lesion of raphe nuclei reduces the concentration of serotonin in preparations of brain microvessels[102]. Furthermore, the neurotransmitter content in capillaries

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may be decreased after destroying nerve terminals with p-chloroamphetamine[102]. However, the presence of binding sites for serotonin in microvascular enriched preparations has not been detected[95]. Therefore, the existence of a functional serotoninergic innervation of brain microvasculature is still controversial.

5) *Other neurotransmitters*

The existence of receptors for histamine in brain microvessels has recently been demonstrated[95,56]. Both H_1 receptors and H_2 receptors linked to adenylate cyclase are present. Histamine seems to be localized in perivascular mast cells and a histaminergic innervation of cerebral microvasculature has not been detected[50].

As regards other putative neurotransmitters, such as GABA, glutamate and peptides, neither the existence of receptor sites nor any physiological function in cerebral microvessel have been reported. However, VIP-sensitive adenylate cyclase has been demonstrated in microvessel enriched preparations, suggesting a role of this neuropeptide in capillary reactivity[46].

Microvascular function in different physiopathological conditions

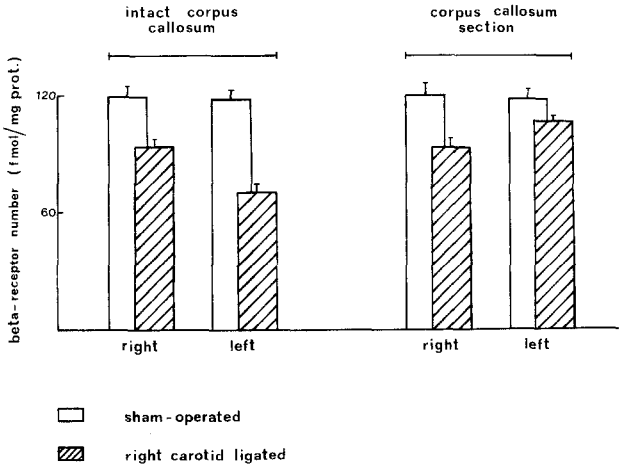
1) *Development and aging*

It is generally accepted that cerebral vascular function is not completely developed during the early periods of the life span [45,51,108]. However, the blood-brain barrier, in young animals, is not completely permeable[11]. In fact, tight junctions are well developed even in newborn animals[51], and saturable carrier-mediated transport mechanisms are present at birth[94]. Large molecule and glucose entrance into the brain in young animals does not differ from that in adults, but certain substances, such as amines, nucleotids and basic amino acids, are transported into the brain by carrier mediated systems faster in newborn than in adult animals, suggesting that the newborn blood-brain barrier is slightly different from that of adults[36,94]. To investigate the basis for these differences, we measured adrenergic receptors in rat cerebral microvessels during development. A lower level of alpha- and beta-receptors was found in early life, which may be correlated with the lower capacity of cerebral vascular regulatory mechanisms observed in this period[60]. It was interesting to note that the ontogeny of beta-receptors in brain tissues is different from that in microvascular tissue[94], suggesting that neuronal and vascular functional developments are not parallel.

The aging process induces relevant modifications in cerebral microvasculature[85], which may be important for the biochemical, physiological and morphological changes observed in the central nervous system. In fact, functional events such as altered cerebral blood flow, reduced oxygen consumption and impaired blood-brain barrier function are reported during aging[85,87]. Furthermore, morphological observations show a loss of capillary endothelial cells and an increase in length and volume of capillaries in aged rat and human brain[5,47,78]. Thinning of capillary walls and declining number of endothelial mitochondria were also described[12]. These changes coincide with adrenergic function alterations observed in microvessels, including decreased content of norepinephrine[29], reduced beta-adrenergic receptor density and lower capacity of the cyclic AMP regenerating system[62]. Such changes during aging for both the neuronal function and the vascular regulation could be of great importance in the clinical aspects related to cerebral dysfunctions in the elderly.

2) *Cerebral hypoxia*

The brain is extremely sensitive to anoxia; in fact, the deprivation of the blood supply induces a failure in synaptic transmission and neuronal death. Both neurotransmitter function and metabolism are altered in cerebral areas after ischemia[76,105,106,115,118,120,121]. These events are accompanied by changes in microvascular function, such as increase in capillary permeability to water and decrease in glucose uptake and O_2 consumption[28,82,112,119]. In view of the possible role of beta-adrenergic receptors in mediating the changes of capillary function in cerebral ischemia, we measured beta-adrenergic receptors in preparations of brain microvessels obtained from gerbils and rats after ligature of the right or left common carotid artery[72]. The results indicate a significant decrease in receptor number in both the ipsilateral and contralateral hemispheres. The data suggest an alteration of adrenergic regulatory mechanisms of brain microvasculature during hypoxia. Surprisingly, in the case of occlusion of the right carotid, the reduction in beta-adrenergic receptor number was more pronounced in the contralateral than in the ipsilateral hemisphere (fig.). The massive release of catecholamines from ischemic neurons may in part account for the observed loss of binding sites; on the other hand, this event does not explain the major reduction of receptor density in cerebral microvessels isolated from the contralateral cortex. It is known that local cerebral ischemia may cause biochemical and functional alterations in brain areas distant from the site of the injury[63,79,111]. In order to investigate the role of nerve connections in biochemical changes observed in the contralateral side of the brain, a transection of the corpus callosum was performed in the rat and beta-adrenergic receptors were measured in brain microvessels after right carotid occlusion. The results (see fig.) indicate that the callosal sec-



Effect of right carotid occlusion on the number of capillary beta-adrenergic receptors in rats with intact or sectioned corpus callosum.

tion partially protects the contralateral hemisphere from neurochemical changes induced by the right carotid ligation[73]. These data may provide evidence that beta-adrenergic receptors located on cerebral microvessels are partially regulated by central adrenergic pathways and that ischemia induces changes in distant areas of the brain in part by a transneuronal mechanism. Furthermore, it is important to note that the impairment of beta-adrenergic receptor function is more pronounced in the left hemisphere also in case of ligature of the left carotid[72]. This event may reflect asymmetries in neuronal regulatory mechanisms of cerebral microvasculature, the clinical relevance of which is at present unknown.

3) *Hypertension and diabetes*

Brain microvessel function and morphology are altered during hypertension. In fact, ultrastructural studies have shown the presence of cerebral capillary injury, such as increased diameter, endothelial degeneration and deposition of collagen, in hypertensive monkeys[32], while physiological evidence indicates an increase in the permeability of the blood-brain barrier[33,42,52,53,71]. In particular, during acute elevation of blood pressure, a disruption of the barrier occurs, resulting in increase in cerebral blood flow, extravasation of markers which normally do not penetrate the barrier and cerebral edema. Increased capillary permeability and cerebral edema are also observed in chronic hypertension[1,86]. In spontaneously hypertensive rats, taken as an animal model of essential hypertension, Palmer showed a reduction in the responsiveness to beta-adrenergic agonists of brain capillary adenylate cyclase[92]. The finding is consistent with the data on decreased beta-adrenergic receptor function, directly measured by radio-receptor binding assay, in brain microvessels prepared from spontaneously and experimental hypertensive rats[74].

These results suggest that functional alterations that occur in hypertension may be partially related to the diminished beta-receptor density in capillaries. The observed impairment of beta-receptor function may be the consequence of an altered pattern of central adrenergic neurons that control brain microvasculature as well as of increased plasma catecholamine levels. The concomitant events of altered neuronal and humoral regulation may be involved in microvascular dysfunction in hypertension.

Diabetes is also reported to affect cerebral microvasculature function. In fact, ultrastructural studies demonstrate an increased cerebrovascular permeability to albumin[113] and an amplified transendothelial transport of pinocytotic vesicles in conditions of severe hyperglycemia[109]. Experiments performed in our laboratories suggest that beta-adrenergic receptors are decreased in cerebral capillaries of diabetic rats[75]. These results may be correlated with the reported data on a decreased activity of beta-sensitive adenylate cyclase in brain microvessels during experimental diabetes[93]. The decrease in beta-adrenergic receptors coupled with the adenylate cyclase system indicate alterations in the adrenergic regulation of cerebral capillaries and may be related to brain microvasculature disturbances occurring in diabetes.

Conclusions

We have presented evidence on a functional innervation of cerebral microvasculature. In addition to metabolic changes and circulating hormones, neuronal pathways are involved in the modulation of cerebral blood flow and vascular permeability. The close coordination between vascular function and neuronal activity may be mediated by neurotransmitter receptors located on brain capillaries.

The complex systems which exist in the brain for the regulation of microvascular function are the result of the extreme sensitivity of cerebral cells to alterations of their environment. Derangements of such systems may have implications in various cerebrovascular disorders.

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Etiology of rheumatoid arthritis

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Summary. Definite genetic associations with immunological cooperative HLA-D(R) antigens have been demonstrated for rheumatoid arthritis (RA). Microbial etiology has not been proven, but some hope for the supporters of this view is still given by small viruses, plasmids of enteric bacteria or perhaps oncogen-like DNA-sequences. Yet, electrophoretical analysis of membrane proteins or surface glycoproteins of RA synovial cells does not show any differences compared to reference cells. Autoimmunity to several tissue elements has been demonstrated, but most of it is of secondary nature. Antigenicities of type II and III collagens are probably only contributory factors for HLA-DR4 positive individuals. Proteoglycans or minor cartilage collagens have not been extensively studied, so far. Endocrine, dietary or psychological influences might be triggering events for otherwise 'preloaded' individuals.
Key words. Arthritis, rheumatoid; cell membranes; etiology; immune regulation; membrane proteins.

Despite the failures to show a single specific cause of rheumatoid arthritis (RA) – perhaps it does not even exist – considerable knowledge has been obtained on the factors associated with the disease. This review aims at summarizing the knowledge available at present from various fields about the onset of RA, where several factors may be acting simultaneously, but with different relative strengths, to precipitate the clinical syndrome.

Genetic disease

Familial aggregation of RA has been thoroughly reviewed by Lawrence[62]. The risk for RA in the first-degree relatives is about two- to three-fold, but it is greatly affected by the severity of disease of the studied subjects: first-degree relatives of patients with seropositive erosive RA have a six to seven-fold excess of bone erosions as compared to the control population, but no excess is noticed in the relatives of seronegative patients. Monozygotic twins of seropositive RA patients have a 33-fold increased prevalence of erosive arthritis compared to the expected value. The figures are consistent with a polygenic inheritance with a threshold in penetrance[62].

In the earlier studies, RA could be correlated neither to ABH blood groups, nor to the transplantation antigens HLA-A, -B or -C, called class I molecules[53]. With the discovery of HLA-D region genes and class II antigens encoded by them, a new genetic link was demonstrated for RA: the allele HLA-D(R)4 was detected in about 46–77% of RA patients, while it occurred in about 14–34% of the control Caucasian population[34, 45, 81]. The relative risk for RA is about four-fold greater in individuals possessing the HLA-DR4 antigen. It is possible that even higher associations will be discovered, when the individual haplotypes of the polymorphic HLA-DR glycoprotein molecules are tested[74]. Also, the high level of IgM rheumatoid factor in the blood of RA patients coexists with the HLA-DR3 allele[81]. The juvenile form of RA differs from the adult disease in its HLA associations[104], indicating a different disease. Seropositivity, female sex and existence of rheumatic relatives tend to correlate positively with the HLA-DR4 appearance[34].

Since none of the genetic parameters correlate with seronegative RA and since that disease is usually milder, it has been postulated that no 'seronegative rheumatoid arthritis' exists, but that those who do not fulfill the classical ARA criteria represent cases of other presently undefined chronic arthritides[14].

Association of RA with other probably immunological diseases has been described: 13% of first or second degree relatives of RA patients have insulin-dependent diabetes and 13% have autoimmune thyreoiditis[110].

The exact biological roles of HLA-D-region coded molecules have not been established yet. At least, they act as stimulators in mixed lymphocyte reactions, as targets for cytotoxic T cells (in addition to the HLA-A, -B and -C molecules) and as controlling elements in antigen presentation[46]. They are expressed by B lymphocytes, activated T lymphocytes and antigen presenting cells (e.g. monocytes, macrophages, dendritic and related cells), i.e. cells involved in the immunological co-operation.

T-lymphocytes of patients with the HLA-DR4 allele react to collagen, while the cells of other patients do not[103]. Additional evidence for the involvement of major histocompatibility complex (MHC) genes in the peripheral arthritis is demonstrated by the strong HLA-B27