CHAPTER 4

THE NEUROIMMUNOLOGICAL INTERACTION IN TERMS OF THE CNA/SAM-AXIS

The noradrenergic system, as one of the two major stress systems, consists, as was seen in the previous chapter, of the central noradrenergic system (CNA-system) and the peripheral sympathoadrenomedullary system (SAM-axis). In this writing the abbreviation CNA/SAM-axis will be used in referring to the noradrenergic system as a whole. In the previous chapter it was shown that activation of the central noradrenergic system and its sympathoadrenomedullary outflow are involved in many behavioural functions and is a feature of almost all arousal states, including physical, emotional and cognitive stress. It has further been linked to a variety of psychological/psychiatric disorders. The aim of this chapter is to provide evidence for the bidirectional interaction between the noradrenergic system and the immune system. This chapter thus represents the neuroimmunological aspects of the psychoneuroimmunological interaction in terms of the CNA/SAM-axis.

Introduction

Despite a vast amount of research on the interaction between the immune system and the noradrenergic system, confusion still prevails. This makes the field rather inaccessible to psychiatrists and physiologists alike. In order to arrive at a relatively rational conclusion, literally hundreds of publications had to be scrutinised. Reductionistic strategies were therefore employed in an attempt to present a simple heuristic representation of the interaction between the immune system and the CNA/SAM-axis. Reductionistic strategies employed included a) presentation of supporting evidence in table, rather than in descriptive format, b) short general discussions, based on the contents of the tables, without superfluous duplication of reference to authors, c) omission, where applicable, of the names of original authors, in favour of review authors, and d) a final summary of the chapter in the form of a schematic presentation of the neuroimmunological interaction in terms of the CNA/SAM-axis. For logistic reasons the immune system is subdivided into the primary lymphoid organs, the secondary lymphoid organs, and the

circulating immunocompetent cells and *in vivo* immunity.

This chapter contains a condensation of what would normally be spread over several chapters. As in the previous chapter a small-scale version of the final schematic integration is presented at the beginning of the chapter (Figure 4.1). The normal size version of Figure 4.1 is presented at its rightful place at the end of the chapter, as Figure 4.4. Presenting the final figure at the beginning of the chapter once again gives the reader, not interested in the detail, the opportunity to get an insight into the bidirectional interaction by merely looking at the final scheme.

The subdivisions include:

Figure 4.1 is presented on the next page, followed by the legend to Figure 4.1 on the subsequent page.

NORADRENERGIC ACTIVITY and IMMUNITY

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he immune system on the noradrenergic activity

Effects on immune system

IN VIVO IMMUNO - REACTIVITY

SECONDARY LYMPH ORGANS

PRIMARY LYMPH ORGANS

PHYCHOSOCIAL DISPOSITION

THYMIC EFFECT ON NORADRENERGIC FUNCTION AND NORADRENERGIC CONTROL CENTRES

1. Necessary for development of neuro-secretory function during early development
2. Maintenance of adrenoceptor density
3. Regulation of adrenoreceptor responsiveness
4. Neuroregulatory effects through thymus-derived cytokines (IL-1, IL-3, MCSF, etc.), thymus-derived hormones (prolactin, ADH, oxytocin, tachykinins etc.) and thymic peptides
5. Behaviour effects include increase in spontaneous behaviour, suppression of anxiety and increased resistance to stress

NORADRENERGIC EFFECT ON SECONDARY LYMPHOID ORGANS (SPLEEN)

1. Involved in prenatal splenic development
2. Regulates splenic perfusion
3. Regulates lymphocyte and granulocyte release from lymphoid tissue in a blood-flow dependent and independent manner
4. Direct role in lymphocyte trafficking, antigen processing and presentation as well as cytokines production
5. Essential for immunocompetence but curtails excessive immunological reactivity
6. Generally immunosuppressive, but may be immune-enhancing, dependent on receptor type, stimulus intensity and duration as well as general immunological status
7. Dual effect within same immunological reaction with stimulation in the inductive phase and inhibition in the effector phase
8. B_2-adrenoceptor-cAMP-protein kinase activity inhibits pro-inflammatory pathway and stimulates anti-inflammatory pathway through differential effects on TH1 and TH2 cells. Can shift immune response from cellular to humoral

NORADRENERGIC EFFECT ON BONE MARROW

Role in perfusion, cellular migration and leukocyte release

BONE MARROW

NORADRENERGIC EFFECTS ON THYMUS

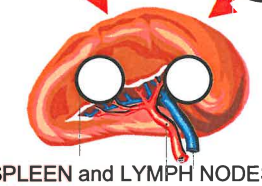
1. Regulatory function in thymus perfusion and release of cells from thymus
2. Necessary for early post-natal development, including thymocyte differentiation and receptor expression
3. Controls thymic micro-environment by influencing intra-thymic neurohormonal and peptidergic secretion
4. Essential for thymus-dependent immunological competence
5. Involved in thymus involution
6. Controls stimulus responsiveness of cells
7. Controls excessive immunological responses
8. May be a factor in gender-dependent differences in immunological responsitivity through interaction with steroid hormones

THYMUS

Thymosins
Thymopeptins
Thymulin
Cytokines
Tachykinins
Neurohypophysial hormones

B-cells

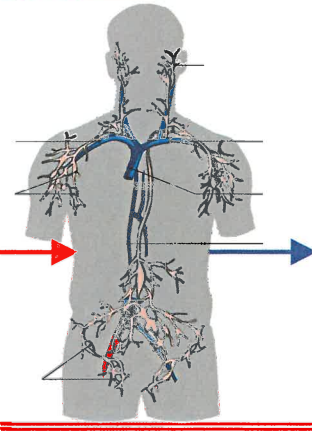
T-cells



SPLEEN and LYMPH NODES

NORADRENERGIC EFFECT ON IN VIVO IMMUNOREACTIVITY

1. Beta-adrenoceptors are found on lymphocytes, monocytes granulocytes and macrophage
2. Alpha-adrenoceptors are found on some immune cells and on vasculature (important in cell traffic and release)
3. Most phagocyte functions appear to be suppressed by NA
4. Essential for T-cell response (cytotoxic T-cell activity, Con A-induced proliferation, IL -2 production, IL -2 receptor expression, T-cell dependent antibody response) but control excessive reactivity
5. Tonic inhibition of tumoricidal activity (NK-cell activity etc) and T-independent antibody responses (polyclonal B-cell responses)
6. Inhibition of macrophage antigen presenting activity
7. Immunological effects of NA stimulation are dependent on receptor type and density, intensity and duration of stimulation as well as on stage of immunological reaction and immunology status
8. Effects on immune responses influence cytokine production
9. Helps to localize inflammatory response
10. Protects body from the tissue damage inflicted by overproduction of systemic proinflammatory cytokines
11. Stress-induced adrenergic stimulation may expose the body to infectious complications and autoimmune diseases and tumour growth
12. Hypoactivity may predispose to fibromyalgia/chronic fatigue syndrome



EFFECTS OF THE IMMUNE SYSTEM ON NEUROLOGICAL FUNCTION.

1. Sensory function
2. Affects neuronal firing rate
3. Affects rate of noradrenaline secretion and stimulates adrenoceptor expression
4. Regulatory feedback through immunocompetent cell-derived cytokines, neuro-endocrine and peptidergic substances
5. Possible antibody – induced cerebral effect
6. Stimulation of the central stress response with a basal tonic inhibitory effect on stress-related behaviour

SUMMARY OF NEUROIMMUNOLOGICAL INTERACTION EFFECTS OF NORADRENERGEC ACTIVITY ON IMMUNITY

1. Necessary for immunodevelopment and immunocompetence
2. Major factor in immunorestraint
3. Essential for self-tolerance (basal tonic inhibitory effect helps to curtail auto-immune reactivity)
4. Opposite effects with acute (enhancement) and chronic (suppression) hyperstimulation
5. Variations in noradrenergic activity implicated in tumourgenesis, chronic inflammatory disorders, stress-induced Immuno-incompetence and allergies
6. Implicated as a factor in the immune alterations obtained through conditioning and in immune alterations with drug abuse

EFFECTS OF THE IMMUNE SYSTEM ON NORADRENERGIC ACTIVITY

1. Necessary for neurosecretory development and maintenance of adrenoceptor expression and functional integrity
2. Acts as sensory system to alert CNS about peripheral infectious and immunological occurences
3. Immunologically-derived cytokines give rise to the central stress response through induction of stress hormone secretion
4. (HPA-axis and CNA/SAM-axis) and inhibition of anti-stress hormones (GH, LH, FSH etc)
5. Immunologically-derived cytokines implicated in behavioural effects such as sickness behaviour, mood changes, sleep, eat and locomotor disturbances, anxiety, as well as exploratory, coping and related behavioural phenomena
6. Excessive immunological inflammatory activity may predispose to psychological disorders
7. Cytokine therapy can have neurotoxic effects partially mediated via central NA system

Figure 4.1: A schematic representation of the bidirectional interaction between the CNA/SAM-axis and the immune system

Legend to Figure 4.1

Figure 4.1: A schematic representation of the bidirectional interaction between the CNA/SAM-axis and the immune system.

[On the left hand side of the diagram the effect of the CNA/SAM-axis on the primary lymphoid organ (thymus and bone marrow) immunity, the secondary lymphoid organ (spleen and lymph nodes) immunity, and on *in vivo* immunological reactivity and competence are shown. The right hand side represents the effects of the immune system on the functional and developmental aspects of the CNA/SAM-axis. At the bottom of the page the overall effects are summarised.]

4.1 Bidirectional interaction between the CNA/SAM-axis and the primary lymphoid organs

4.1.1 Effect of the CNA/SAM-axis on the primary lymphoid organs.

Its as yet impossible to be dogmatic about the interaction between the CNA/SAM system and the primary lymphoid organs, as

- the results of *in vitro* tests do not represent a good reflection of occurrences in the *in vivo* environment
- a multitude of factors influence the microenvironment, and by implication the functional integrity, of the primary lymphoid organs
- the interaction is dependent on the chronological order of neural and immune stimulation,
- previous immunological sensitisation, and on the duration of the heightened noradrenergic stimulation
- across-species extrapolation of results may sometimes lead to confusion

Investigating the effect of adrenergic function on the vascular and blood flow behaviour of the thymus is fairly simple in comparison to that on the cellular elements. This is especially true when referring to the mechanisms of negative and positive selection of T-cells, which form part of the differentiation process of bone marrow derived thymic cells. Maturation and differentiation of thymic cells are known to be influenced both by extra-thymic neuroendocrine factors and by the microenvironment of the thymus itself. The microenvironment of the thymus is however, also affected by the neuroendocrine regulatory mechanisms. The regulatory role of neuro-endocrinological substances on the thymocytes, be it direct or through an influence on the microenvironment, is a two-way street as humoral substances from the thymus are in turn able to influence extra-thymic endocrine secretion. These interactions are touched upon in the following paragraphs.

The microenvironment of the thymus is largely a product of the secretory ability of the thymic epithelial cells, and of the cellular interactions mediated through adhesion molecules and membrane receptors (1). Cell-to-cell interactions are probably also regulated by humoral factors. The epithelial cells of the thymus are known to secrete:

- peptide hormones such as the thymosins, thymopeptin and thymulin (1,2,3). Thymosins, such as thymosin-α_1, thymosin β_4 and thymosin factor 5, exert functions such as the induction of lymphopoiesis, stimulation of T-cell maturation, maintenance of T-cell reactivity as well as augmentation of mitogen responses, alloreactivity and lymphokine production (4,5).
- cytokines such as interleukin-1, interleukin-3, interleukin-6, as well as the granulocyte macrophage colony stimulating factor, (6,7,8)
- intracellular matrix proteins, including type IV collagen, laminin and fibronectin (9) and probably a number of hormones not generally associated with the thymus, such as prolactin, antidiuretic hormone, and oxytocin (1,10). Thymic epithelial cells are said to synthesize neuroendocrine-related peptides belonging to the 3 neuroendocrine families i.e. the neurohypophysial, the tachykinin and the insulin families (11).

The above factors all contribute to the microenvironment of the thymus - directly as well as indirectly through their effects on thymocyte proliferation, maturation and differentiation and thus by implication, to thymic immune function.

The secretory function of the thymic epithelial cells, and therefore the micro- environment of the thymus, is further known to be influenced by the so-called developmental hormones of the circulation, such as the thyroid hormones, insulin, glucocorticoids, growth hormone and prolactin. Evidence for their role in thymic function is seen in

- the expression of their receptors on thymic epithelial cells
- their modulation of thymic hormone secretion, of cytokeratin expression and of cellular proliferative processes (12,2)
- in patients with hormonal disturbances where the stimulatory effects of hormones such as the thyroid hormones, insulin and growth hormone are borne out by changes in thymic hormone levels in patients with hypopituitarism, acromegaly, hyper- and hypothyroidism, low T_3 syndromes and in patients with type 1 diabetes mellitus (10).

Prolactin is perhaps the major neurohypophysial hormone with regard to thymic function. Prolactin has even been suggested to be the determining factor in thymic immunological competence (1).

Immunohistochemical studies further demonstrate an abundancy of peptidergic innervations and peptidergic receptors in the thymus and indications are that neuropeptidergic modulation is involved in alterations of the immune response (13,14,8). Reported thymus-associated peptidergic activities include vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP) and substance-P (SP). The existence of co-localisation of various transmitter substances within a single nerve fibre has also been reported. In view of co-localised monaminergic and peptidergic transmitter substances, such as noradrenaline and substance P, a functional modulatory co-operation is suggested between the sympathetic nervous system and the influence of peptide hormones on thymic immune function. This presents yet another confounding factor in the attempt to define the role of one single influence, such as the noradrenergic system, on primary lymphoid organ immunity.

Publications dealing with the effects of the CNA/SAM system on thymus immune function are summarised in Table 4.1. The results of various workers are presented in table form in an attempt to gain some insight into this field, where a marked degree of confusion still exists.

Table 4.1 : Noradrenergic influence on primary lymphoid organs

ORGAN	INNERVATION	TECHNIQUE	FUNCTIONAL IMPLICATIONS	REFERENCES
BONE MARROW	Myelinated and unmyelinated fibres in bone marrow	Light and electron microcopy	Innervation of bone marrow	Calvo, 1968 (15)
	Fibres found in association with vascular plexuses and marrow substance	Fluorescence microcopy	Possible role in hemopoiesis and cell migration through blood flow effects	Felten et al, 1985; Felten and Felten 1991 (16,17)
	Innervation of bone marrow starts late in foetal life - just before the onset of haemopoietic activity	Microscopy	May be involved in blood flow, cellular migration and release	Calvo & Haas, 1969; Miller & McCuskey, 1973 (18,19)
	Nerve endings among lymphopoeitic and haemopoietic cells and along vasculature	Immuno-histochemistry	Possible influence on haemopoiesis and perfusion	Gibson-Berry et al, 1993 (20)
	Sympathectomy and a_1-adrenergic antagonist increase peripheral blood leukocyte count	Chemical sympathectomy with 6- hydroxydopamine	A role for NA innervation in thymic perfusion and leukocyte release	Maestroni et al, 1992 (21)
	Controversy as to whether sympathetic fibres have any synapses with cells, and thus any direct influence on cell development and release	Silverstaining Falck – Hillarp histofluorescence	Speculation that effects on cell release may be secondary to effect on peripheral resistance or effect via volume transmission	Takase and Nomura 1957; Muller and McCuskey 1973; De Pace and Webber, 1975 (22,19,23)
	NA release mobilizes fat from the marrow			Tran et al, 1987 (24)
	Prozasin (a_1-antagonist) increases peripheral leukocyte count and may possibly increase myelopoiesis. Same effect with chemical sympathectomy	Pharmacological manipulation with prozasin and with 6-hydroxydopamine	May be just a blood flow phenomenon	Maestroni et al, 1992 (21)
THYMUS	The thymus can correct the brain alpha and beta-adrenoceptor decrease in density which occurs in old age	Neonatal thymus graft	There is a two-way effect between CNS and thymus. Thymus supports CNS receptor density	Rossolini et al, 1991 (25)

Mature thymocytes (= cortisone resistant) express twice the number of beta-adrenoceptors per cell as immature (cortisone sentitive) thymocytes	Ligand binding studies	Innervation, as for the thymus as a whole, increases with maturation	Fuchs et al, 1988; Bach 1975 (26,27)
NA innervation predominantly vascular in early development. Vascular innervation increases with growth	Electron microscopy; Fluoressence immunochemistry	A role in thymic perfusion	Bulloch and Pomerantz, 1984; Ackerman et al, 1991, Singh, 1984; Williams and Felten 1981 (28,29,30,31)
NA innervation of the thymic parenchyma is predominantly a post -natal occurence	Fluorescence histochemistry	Suggests a very low or no role of the NA system in foetal development	Ackerman et al, 1991; Bellinger et al, 1992 (29,8)
The cortical zone is the first parenchymal area to be innervated followed by a dramatic increase in the innervation of the corticomedullary junction	Fluorescence histochemistry		Ackerman et al, 1991 (29)
NA fibres to the thymus are derived predominantly from post ganglionic cell bodies of the superior cervical ganglion and stellate ganglion	Fluorescence histochemistry	Confirmation of sympathetic nervous system innervation of thymus	Bulloch and Pomerantz, 1984; Tollefson and Bulloch, 1990 (28,32)
In the adult a network of NA fibres in association with blood vessels, arterial and venous plexuses, intralobular septa and distributed amongst thymocytes of subcapsular, corticals cortico-medullary and medullary parenchyma. Cortical parenchyma (immature thymocytes) well innervated. Cortico-medullary boundry (important for thymocyte emigration) densely innervated. Peak parenchymal density occurs after involution. Increase in embryogenic innervation matched by an increase in thymic NA concentration. Noradrenergic supply to medulla limited mainly to bloodvessels	Ultra structural microscopy, Fluorescence histochemistry, TH^+ immunocytochemistry. Liquid chromatography with electrochemical detection (HEC)	A role for NA in vascular resistance, blood flow and perhaps thymocyte migration, within as well as from the thymus A possible role for NA in thymocyte proliferation and maturation Peak density after involution may merely reflect the decrease in thymic size	Ackerman et al, 1991; Bellinger et al, 1988; Bellinger et al, 1990; Felten et al, 1985; 1987; Williams and Felten, 1981; Williams et al, 1981; Felten & Felten 1991; Madden et al, 1995; Bulloch et al, 1987 (29,33,14,16,31, 34,13,35,36)
Thymocytes in early developmental stages demonstrate higher cAMP responses to NA stimulation than that during later developmental stages	Equilibrium binding assays	May be a role for NA in early cellular developmental processes OR a function of lower receptor density and NA availability	Singh,1979 (37)
Thymocyte maturation is accompanied by an increase in ß-adrenoceptor density (especially in cortex and paracortex) and a decrease in 2nd messenger responsiveness to NA stimulation	Equilibrium binding studies LCEC for monoamines	May be a role for NA in differentiation of thymic cells. May be a functional down/up regulatory mechanism	Bellinger et al, 1989; Ackerman et al,1991; Fuchs et al,1988; Bach, 1975 (38,29,26,27)
Mature spleen cells show a smaller cAMP response to isoproterenol than mature adult stage thymocytes	Equilibrium binding studies	Possibly further prove of a decreased responsiveness with maturation. or of a up/down regulatory mechanism due a change in receptor density of NA c oncentration	Bach 1975 (27)
NA fibres are maintained with aging - accompanied by an increase in thymic NA concentration. An appearance of hyperinnervation with thymic involution	Fluorescence histochemistry. LCEC for monoamines	May be secondary to thymic involution (thymus thus shows slower NA fibre development but more prominent maintenance of fibres in adulthood than secondary lymphoid organs	Ackerman et al, 1991 (29)
ß-adrenergic receptors shown on thymocytes	Mostly receptor-ligand binding assays	Indicates a role for sympathetic system in immunity	Fuchs et al, 1988; Singh 1979; Singh, 1984; Singh and Owen,1976; Singh et al, 1979 (26,37,39,40)
Rat thymus contains predominantly $ß_2$-adrenoreceptors (78%). Upregulation is seen under influence of sex steroid hormones	Radio-ligand binding studies	Suggests gender associated NA modulation of cell mediated immune responses. Could be of significance in gender-dependent immune responses	Marchetti et al, 1990 (41)

Thymic $ß_2$ adrenergic receptors show a clear sexual dimorphism in receptor organization during sexual maturation. Receptor density decreases with castration and increases when castrated rats receive oestradiol	Autoradiography	$ß_2$-adrenoreceptors density in the thymus is modulated by steriod hormones through transcriptional control of $ß_2$-adrenoceptor gene expression	Marchetti et al, 1990; Marchetti and Labrie 1990 (42,43)
Adrenaline increases thymocyte proliferation into the metaphase in a time and concentration dependent fashion	Tissue culture preparation	Suggests increased proliferation with B_2 -stimulation (The process is stimulated by phosphodiesterase inhibitors)	MacManus et al, 1971 (44)
Increases in intracellular cycle AMP augments differentiation and maturational marker expression (Thy-1) of thymic stem cells. This process is modulated by thymic hormonal factors. May act synergictically with thymopoeitin	Tissue culture preparations	cAMP promotes cellular maturation and proliferation. NA raises cAMP concentration and would thus by implication stimulate differentiation and maturation	Scheid et al, 1973; Scheid et al, 1975; Singh and Owen,1975; Singh and Owen, 1976 (45,46,47,39)
Thymus-dependent immune function and hormonal activity is suppressed in the absence of NA innervation	Thymic grafts	NA necessary fot T-cell dependent immune function	Bulloch et al, 1987 (36)
Severence of the sympathetic supply to the thymus suppresses the proliferative response to footshock and mitogens	Surgical sympathectomy	NA stimulation necessary for thymic proliferative response to stimulation in adult rodents	Wan et al, 1993 (48)
Chemical denervation of the thymus supports thymocyte proliferation in the thymic cortex but induces weight loss and apoptosis of the thymus	Chemical denervation with 6-hydroxydopamine or quanethidine	Suggests that NA inhibits cellular proliferation in thymus. Secretion of other humoral factors are however also suppressed by 6-hydroxydopamine	Kendall-Al-Shawaf, 1991 (49)
NA innervation suppresses thymocyte proliferation and differentiation in grafts	Foetal thymic transplantation (may represent chronic effect)	Sympathetic system suppresses proliferation and inhibits differentiation	Singh, 1985a; Singh,1985b (50,51)
Neuropeptide Y associated with NA fibres	Microscopy	Perhaps a functional modulation of NA function by neuropeptide Y	Kendall and Al-shawaf, 1991 (49)
The immune response induces marked time dependent changes in ß-adrenoceptor numbers and distribution as well as in the cAMP response	Radio-ligand binding studies, Autoradiography Northern blot	The NA activity should always be interpreted in the light of any antigenic stimulation as it may trigger an up of down regulation of $ß_2$-gene expression	Morale et al, 1992 (52)
Acute immobolization stress increases $ß_2$-adrenoceptor binding sites on thymocytes in a time-of-stress-application dependent manner. Chronic stress does not show the same effects	[3H] - DHA specific binding	Result of receptor density should be analysed as a function of psychological state and exposure time	Kuberu et al, 1992 (53)
Restoration of $ß_2$-adrenergic responsiveness in the brain of old mice and of young athymic mice	Neonatal grafts. Thymic extract (TME) administration	Some humoral thymic factor affects adrenergic Receptor responsiveness	Rossolini et al, 1991 (54)
The thymic extract (TME) modifies the isoproterenol- induced increase in submandibular gland and brain cortex DNA synthesis. TME increases a_1-adrenoceptor density	Grafts, TME administration	Some thymically- derived molecules can increase α_1-adrenoceptor density	Basso et al, 1994; Rossolini et al, 1991 (55,54)
Antigenically induced lesioning of the locus coeruleus promotes thymus involution and $CD4^+$ lymphocyte depletion of blood and suppresses the Arthus and delayed skin reactions	SRBA as antigen. Tuberculin as antigen	The sympathetic nervous system, by implication, contribute to thymic involution	Jankovic et al, 1994; Nikolic et al, 1993; Jovanova - (56,57)
Thymic sympathectomy enhances thymosin α_1 production	6-Hydroxydopamine (6OHD)	The sympathetic system suppresses the secretion of thymosin α_1 from epithelial cells	Hall et al, 1982 (58)

From published reports (see Table 4.1 for references) it can be deduced that

- Noradrenergic innervation of endothelial cells, epithelial cells, thymocytes, macrophages and mast cells have been established beyond doubt. Most of the noradrenaline found in the thymus appears to be of noradrenergic fibre origin, rather than being derived from the

circulating catecholamine pool.

- From ontogenic studies the adrenergic innervation would appear to be of little importance for prenatal thymic development as innervation is predominantly a postnatal development.
- The first thymic effects during development are probably that on vascular diameter and thus on blood flow.
- The first parenchymal innervation during development is seen in the cortical zone that generally contains the immature thymocytes. This may imply that the sympathetic system is of importance for the early development of thymocytes and is supported by the reported positive catecholamine influence on differentiation and receptor expression. The second parenchymal area to develop adrenoceptor functionality is the cortico-medullary junction, which would suggest a role for NA in thymocyte migration.
- From published data it is conceivable that α_1-adrenergic receptors predominate on vascular and capsular elements, which would support the suggestion of an adrenergic control of thymus perfusion and, by implication, in cell traffic.
- Adrenoreceptors on the thymocytes and other parenchymal elements are predominantly $ß_2$, which is common for most metabolic/secretory adrenergically induced functions in the body. A variety of factors such as the sex steroids seem to be able to bring about an up or a down regulation in adrenoreceptor density and in the stimulated c-AMP response. Too little is, however, known about the context, in terms of other factors of the thymic microenvironment in which these alterations occur, in order to come to any conclusion for the thymus *per se*.
- An increase in adrenoceptor density with a concomitant suppression of the stimulus responsiveness would appear to accompany the maturation of thymocytes. Whether this phenomenon reflects part of the maturation process, or whether it is merely a function of noradrenaline availability is not yet clear.
- The apparent positive effect of NA on thymocyte early development, differentiation and on receptor expression as well as the suppression of thymic hormonal activity in the absence of NA, may point toward a role for the sympathetic system in enhancing thymus-dependent immunity during the early stages.
- Despite thymic innervation being predominantly a postnatal development, the innervation is, in contrast to other lymphoid organs, maintained for a longer period than that of the spleen - with peak density found during thymic involution. This might merely

be a reflection of the shrinkage of the thymic mass, without a concomitant decrease in the number of adrenergic fibres. However indications, are that the sympathetic system might be involved in thymic involution as lesioning of the locus coeruleus promotes thymic involution.

- Integrating the implied stimulation on early development, the possibility that NA may be involved in eventual thymic involution, would suggest both a developmental and an inhibitory role for the sympathetic nervous system in the cellular immune system.
- It would further appear as if sympathetic stimulation may have an inhibitory effect on the thymic epithelial cell secretory function as thymosin α_1 increases after pharmacological sympathectomy.
- Thymic extract has in turn been shown to increase cerebral α_1-adrenoceptor density.
- The localisation of monoaminergic and peptidergic substances in the same fibres points towards the modulation of sympathetic activity by other neurotransmitters.
- Certain interactions such as the effect of thymic hormones on the maintenance of adrenergic responsiveness have been shown. A functional interdependency is also known to exist between adrenergic activities on the one hand, and steroid hormones, as well as a number of thymic humoral substances.

It is clear that a multitude of substances are involved in the immunology of the thymus and that it is virtually impossible to decipher the role of any individual factor. A further confounding factor in establishing the effect of the sympathetic system on thymic function is that very few of the workers tried to distinguish between acute and chronic noradrenergic influences. This is important as it is well known that acute and chronic stress-induced neuroendocrine alteration of the immune system may differ.

In conclusion it appears reasonable to assume that the CNA/SAM-axis affects thymic immune functions

- by regulating postnatal thymic perfusion and thus also cellular migration and maturation
- by being a factor in the control of the microenvironment of the thymus
- by being necessary for normal thymic developmental immunocompetence but
- by also being involved in the control of excessive immunological responses and in thymic involution

4.1.2 Effect of the primary lymphoid organs on the noradrenergic system.

It would appear that the primary lymphoid organs, and in particular the thymus, might have a positive effects on the nervous system. Very little is known about the effect on the CNA/SAM system *per se*. It has however been shown that thymic grafting can restore ß-adrenergic responsiveness and that thymic extracts can increase α_1-adrenoceptor density. This increase in density was however seen to be accompanied by a decrease in receptor-mediated responsiveness (55). Whether these two effects occur simultaneously, and whether it has any functional implication is not clear.

However, indications are that the thymus may be necessary for the maturation of the neuroendocrine system during early life and that it might perhaps also play a role in the neuroendocrine secretory functions of the adult brain. This is substantiated by the fact that neuroendocrine abnormalities are known to result from either the congenital absence of the thymus or from neonatal thymectomy. (59,60) A possible regulatory role on neuroendocrine function is further seen in the effect of thymus-derived immunoregulatory substances on neuroendocrine function (61). See Table 4.2.

Table 4.2: Influence of thymus-derived substances on the neurological system

THYMIC FACTOR	EFFECT ON NERVOUS SYSTEM
Thymosin $ß_4$	Stimulates hypothalamic-pituitary-gonadal axis in vitro
Thymosin α_1	Stimulates hypothalamic-pituitary-adrenal axis in vivo
Thymosin F_5	Stimulates hypothalamic-pituitary-adrenal axis in vivo
Lymphokines	Decrease hypothalamic noradrenaline
Lymphokines	Stimulate glial cells
Interleukin 1	Stimulates hypothalamic thermoregulatory centres
Interleukin 1	Stimulates slow-wave sleep
$C3_a$	Modulates feeding behaviour at level of hypothalamus
ACTH	(Neural and pituitary peptide also produced by lymphocytes)

ß-Endorphin	(Neural and pituitary peptide also produced by lymphocytes)
Interferon	Induces lethargy and depression

Adapted from Hall et al, 1985 (61). See references in 61.

The thymic effects on the nervous system would appear to decline with aging. This apparently results from the combined effects of the age-associated thymus involution and a desensitisation of the neuroendocrine tissues to thymus-derived immunotransmitter substances.

4.1.3 Summary on the bidirectional interaction between the CNA/SAM-axis and the primary lymphoid organs.

In conclusion it can be said that

- the neuroendocrine system would appear to be essential for the ontogenic development of the thymus
- the thymus is in turn necessary for the early development and maintenance of the neuroendocrine system
- a bidirectional influence exists between the thymus and the neuroendocrine system with regard to secretory and other cellular functions
- although the nervous system is essential for the development of thymus-dependent immunocompetence, it is also involved in restraining unwarranted cellular immunity and in thymic involution
- the adrenergic influence on thymic function should not be seen as a major independent effect but rather as one aspect of the neuroendocrine, immunotransmitter milieu that regulates thymus function.

The interaction between the primary lymphoid organs and the CNA/SAM-axis is summarised on the next page in Figure 4.2.

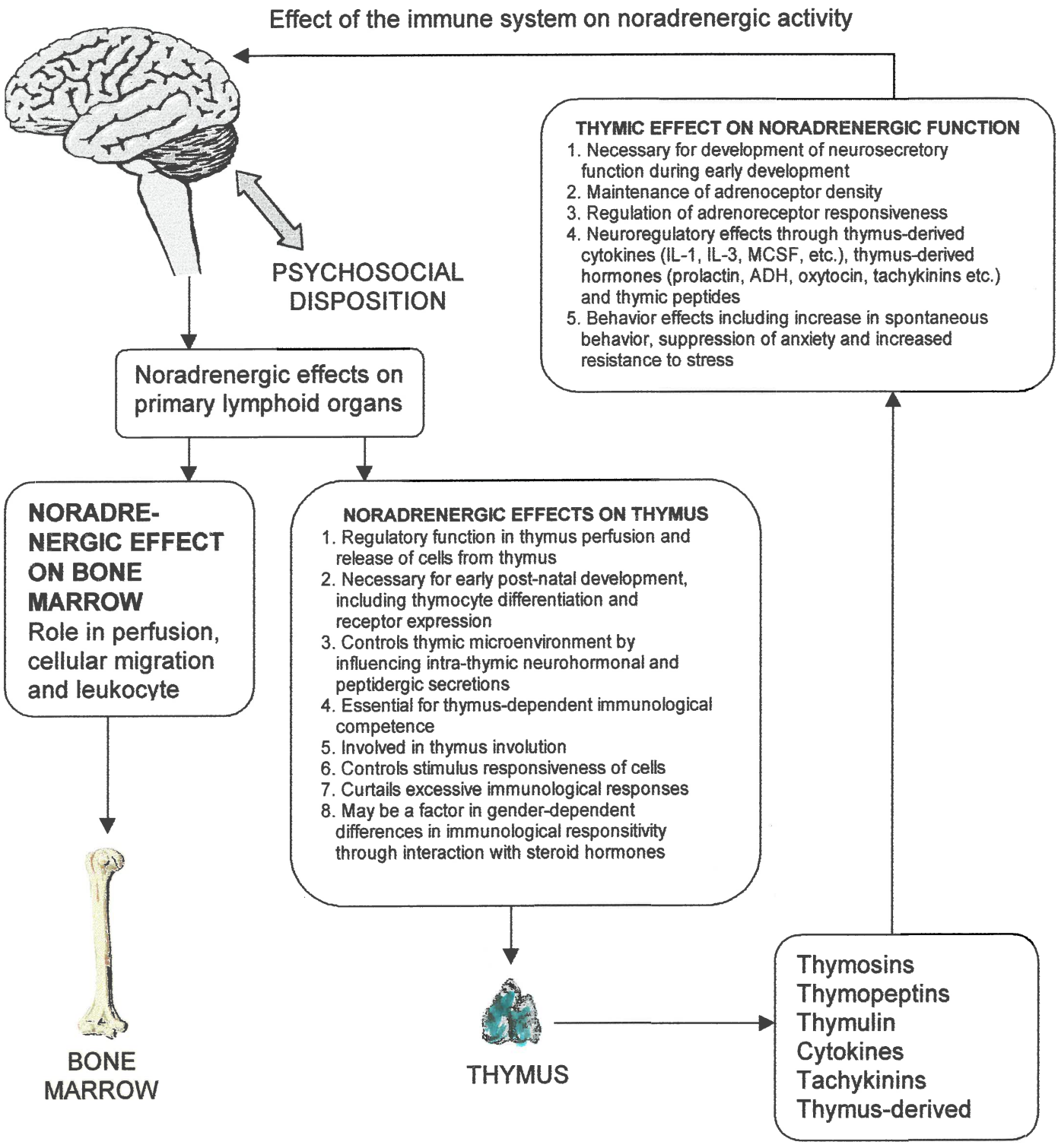


Figure 4.2: Bidirectional interaction between the primary lymphoid organs and the CNA/SAM-axis

4.2 Bi-directional interaction between the CNA/SAM-axis and the secondary lymphoid organs

4.2.1 Effect of the CNA/SAM-axis on the secondary lymphoid organs

A comprehensive discussion, including all the work done on the adrenergic influence on secondary lymphoid organ immunity is beyond the scope of this writing. The results of representative publications dealing with the innervation and some of the major findings on the influence of the noradrenergic system on secondary lymphoid organs are summarised in Table 4.3.

Table 4.3: Noradrenergic influence on secondary lymphoid organs

ORGAN	OBSERVATION	TECHNIQUE	FUNCTIONAL IMPLICATION	REFERENCES
SPLEEN	1.NA fibres along splenic artery, central artery and its branches, the peri-ateriolar lymphatic sheath (PALS), and in assoiation with capsular and trabecular systems and in splenic parenchyma (marginal sinus, the marginal zone, parafollicular zone)	Electron microscopy Fluorescence histochemistry Isolated perfused human spleen Double-lable immunocyto-chemistry	Early findings on vascular and capsular innervation point towards a role in smooth muscle contraction and thus resistance to blood flow	Ader et al, 1990; Felten et al, 1987; Felten et al, 1987; Felten and Felten 1992; Felten et al, 1992; Williams and Felten 1981; Ackerman et al, 1991. (64,65,66,67,17, 31,29)
	2.No innervation of B-cell follicles could be shown with fluorescence histochemistry. Single fibres into follicles could only be shown by double lable immunocytochemistry	Double lable immuno-cytochemistry Fluorescence histochemistry	Sympathetic innervation of follicles is minimal or absent	Felten et al, 1987 (65)
	3.Fibres in red pulp mainly in areas adjacent to trabecular and venous plexuses	Fluorescence histo-chemistry	Sympathetic effect predominantly On blood flow?	Felten et al, 1985 (66)
	4.Cell bodies of post ganglionic fibres to spleen situated either in the paravertebral ganglia or coeliac-superior mesentric ganglionic complex		Indicates innervation pathway	Bellinger et al, 1989 Nance and Burns, 1989 (38,68)
	5.TH^+ nerve fibres in pposition to $CD19^+$ T-cells, T-helper cells and T-cytotoxic cells of the PALS, the $CD19^+$ T-cells and $SIgM^+$B-cells of the para-follicular zone as well as the $ED3^+$ macrophages and IgM^+B-cells of the marginal Sinus	Double-lable immuno-cytochemistry	Direct contact exists between SAM axes and splenic cells – Evidence for neural immune interaction at splenic cellular level	Felten et al, 1987a Felten et al, 1987b Felten et al, 1992 (65,66,67)
	6.Close association between fibres and cellular elements of white pulp	Electron microscopy	Cryptocine arrangement	Galindo and Imaeda, 1962 (69)
	7.Direct close apposition between NA nerve terminals and lymphocytes and macrophages of the white pulp appositions are closer than smooth muscle synapses or neuro-effector junctions	Immunocytochemistry (TH^+ specific antibodies)	Imply direct influence for the sympathetic system on splenic lymphocytes and macrophages	Felten and Olschowka, 1987 Felten et al, 1987b (70,66)
	8.Innervation declines with age in parallel with a decline in the number of splenic lymphocytes and macrophages	Fluorescence histo-chemistry	In contrast to thymus where no decrease occur	Ackerman et al, 1991 (29)
	9.Destruction of NA fibre terminals all but completely clear the spleen of NA	Neurotoxin destruction or/and ganglionectomy HPLC	The majority of splenic NA is of NA fibre origin	Bellinger et al, 1989 Felten et al, 1987b Williams et al, 1981

			(38,66,34)
10.More common to find innervation in association with T-dependent than with B-dependent areas. Areas which contain mixed cells i.e. T-lymphocytes, B-lymphocytes, macrophages, etc. are largely innervated by noradrenergic/ NPY containing fibres	Histochemistry	More significant influence of NA on the cellular than humoral immunity	Felten et al 1987a+b (65,66)
11.Splenic diameter decreases with intravenous administration of α_1-adrenergic stimulants (dogs)	Adrenergic stimulants Sonomicrometry	Splenic contraction is mediated through α_1-adrenoceptor activity	Ojiri et al, 1993 (71)
12.The suppressive effect of morphine on conA-stimulated proliferation of splenic lymphocytes is mediated through macrophage derived nitric oxide	Pharmacological manipulation of lymphocyte functions	Splenic macrophages are innervated by adrenergic fibres but effects often indirect	Fecho et al, 1994 (72)
13.The majority of splenic adrenoceptors belong to class B_2 and are found in the capsule, marginal zone of red and white pulp with low densities in the white pulp	[125] cyanopindolol autoradiography	It is suggested that beta-adrenoceptors are present on mature splenic cells and not likely to be involved in homing mechanisms	Fernandez-Lopez et al, 1994 (73)
14.Increase in lymphocyte and granulocyte release from the spleen after NA and ISO injection. Blocked by both phentolamine and propanolol without a change in blood flow	Pharmacological manipulation	Release not a mere blood flow-induced phenomenon. Both α and ß-adrenoceptors involved	Ernström and Sandberg, 1973 Ernström and Soder, 1975 (74,75)
15.The SNS inhibits antigen processing/presentation and T-helper cell response	in vivo	Immune suppression by SNS	Heilig et al, 1993 (76)
16.Propanolol suppresses the conditioned prevention of adjuvant-induced arthritis	Pavlovian conditioning	The sympathetic suppressor effect on splenic proliferation is mediated through ß-receptors	Lysle et al, 1991 (77)
17.Propanalol blocks the conditioned suppressive effect on splenic mitogen responsiveness	Pavlovian conditioning	Conditioned immune alterations of splenic lymphocytes are mediated through ß-receptors	Lysle et al, 1991 (77)
18.The alpha adrenoceptor antagonist phentolamine blocks the cold stress augmentation of mitogen - induced splenic lymphocyte IgG and IgM production. Beta blockers enhance the effects of cold stres	Pharmacological intervention	Both alpha and beta effects on the immune response and the two may directly oppose each other	Carr et al, 1993 (78)
19.Pretreatment with a non-selective ß-adrenergic antagonist, a $ß_1$ antagonist as well as a $ß_2$ antagonist prevents the suppressive effect of morphine on mitogen induced proliferation of splenic cells	Pharmacological manipulation	ß-adrenoceptors are involved in the immune suppressive effect of morphine	Fecho et al, 1993 (79)
20.Suppressor T-cells have the highest density, cytotoxic T-cells and intermediate density and helper T-cells the lowest drenoceptor density. Splenic ß-cells (in mice) express twice the number of ß-adrenoceptors than T-cells	Ligand binding	1.May be a noradrenaline up or down regulation 2.ß-cells may be influenced by NA from adjacent areas	Kahn et al, 1986 Van Tits et al, 1990 Kobilka, 1992 Fuchs et al, 1988 (80,81,82,83)
21.Spleen cell adrenoceptor density decrease with immunisation	SRBC immunization	Lymphocyte activation changed (α) ß-adrenoceptor density	Fuchs et al, 1988 (83)
22.NA and ISO enhances LPS induced proliferation and differentiation of splenic lymphocytes	in vitro exposure	ß-effect (blocked by propanolol)	Kouassi et al, 1988 (84)

LYMPH NODES AND LYMPHOID TISSUE OF GIT	1.NA fibres enter lymphnodes at hilus, run along vasculature, distribute in medullary cords amongst mixed populations of lymphocytes and macrophages in subcapsular regions spares innervation of ß-lymphocyte follicles	Fluorescence histochemistry Double lable immunocyto-chemistry	Same general pattern as spleen	Ackerman et al, 1987 Felten et al, 1987b Felten et al, 1984 Felten et al, 1981 Felten et al, 1992 Giron et al, 1980 Madden and Felten, 1995 (85,66,86,87,88, 89)
	2.In lymph nodes NA fibres found within subcapsular zone, paracortical regions, cortical regions, medullary cords. No obvious innervation of ß-cell follicles	Fluorescence histo-chemistry Double-lable immunocyto-chemistry	Same general pattern as spleen	Felten and Felten, 1991 Felten et al, 1987a Giron et al, 1980 Madden and Felten, 1995 (77,65,88,89)
	3.In rodents NA fibres to cervical, mesentric and popliteal lymph nodes. Decline in number and activity as a function of age	Fluorescence histo-chemistry	Possibly a concomitant decrease of NA Innervation/function and cell-mediated immune function. Corresponds to spleen. Opposite from thymus	Bellinger et al, 1989 Madden and Felten, 1995 (38,89)
	4.Substances which raise intracellular C-GMP augment the release of lymphocytes	Administration of serotonin and substance P	Multiple influences on release of lymphocytes from lymph nodes	Moore, 1984 (90)
	5.Infusion of dbcAMP decreases lymphocyte release. Infusion of dbcGMP increases lymphocyte release	Chemical sympathectomy	The autonomic system should by implication thus be able to regulate lymphocyte release directly – not a blood flow phenomenon	Moore and Lachman, 1982 (91)
	6.Sympathectomy enhances the collection of lymphocytes in lymphnodes and decreases the migration of lymphocytes from lymphnodes	in vitro - isolated lymph-node perfusion	An inhibitory effect on circulating lymphocyte numbers	Madden and Livnat, 1991 (92)
	7.The primary antibody response in sympathectomized animals varies from unaltered, to enhanced to suppressed	6-hydroxydopamine	6-hydroxydopamine sympathectomy has wider neural effects and fibre regrowth may occur. Adrenal medullary catecholamines may effect intact receptors	Miles et al, 1981 Besedovsky et al, 1979 Hall et al, 1982 Madden and Livnat, 1991 (93,94,95,92)
GALT (Gut associated lymphoid tissue)	1.NA fibres of GIT lymphoid tissue distributed through T-dependent zones and in lamina propria. No evidence of ß-lymphocyte follicle innervation	Electron microscopy Fluorescence histo-chemistry, etc.	Corresponds to other secondary lymphoid tissue. Indicates a direct influence of sympathetic nervous system on lymphocyte trafficking, antigen processing and presentation as well as on T-cell function, B-cells maybe affected by sympathetically altered T-cell functions such as altered cytokine production	Felten and Felten, 1991 Felten et al, 1987 Giron et al, 1980 (17,65,88)

SNS = Sympathetic nervous system; NA = noradrenaline; GIT = gastro-intestinal; SRBC = sheep red blood cell; LPS = lipopolysaccharide

The following discussion is based on publications referred to in the table above and on a number of publications of which the references appear in the text.

In contrast to the thymus, i.e. to primary lymphoid tissue, noradrenergic innervation of the secondary lymphoid organs is present during the early developmental periods and declines with age. The noradrenergic innervation of the spleen appears to precede the development of the cellular elements, which would imply a role for the sympathetic nervous system in the developmental and maturational processes. An age-dependent decline in the adrenergic innervation would appear to parallel the decrease in the number of splenic lymphocytes and macrophages.

The distribution of noradrenergic innervation to secondary lymphoid organs implies a direct role for the sympathetic nervous system in mechanisms such as lymphocyte trafficking, antigen processing, antigen presentation and as a result, T-lymphocyte functional integrity. B-cell function would, if affected, be influenced, either indirectly by noradrenaline-induced changes in T-cell and macrophage activity or by cytokine production, or directly by noradrenaline, diffusing from fibres, not in direct contact with B-cells.

It is clear that the CNA/SAM-axis effect on the spleen and other secondary lymphoid organs depends on

- the adrenergic receptor type
- the concentration of noradrenaline
- the receptor density
- the presence of peptidergic innervation
- the presence of cytokines
- the cell-type involved in the immune response
- the antigenicity of the stimulus
- the time of adrenergic stimulation relative to the antigen stimulation
- the duration of the adrenergic stimulation (acute or chronic)
- the activation state of the immune cells at the time of noradrenergic stimulation.

The reader is referred to Table 4.3 and to a number of reviews for more details on the effects of the above-mentioned factors on the adrenergically –induced alterations in the secondary lymphoid organ immune response (62,35,63 29,8).

4.2.2 Summary of the bidirectional interaction between the CNA/SAM-axis and the secondary lymphoid organs.

From Table 4.3, and in considering from the number of factors that may be influential in determining splenic immune reactivity in response to adrenergic stimulation, it is evident that the sympathetic influence can't summarily be described as either inhibitory or stimulatory. Probably the best, yet vastly oversimplified, way to summarise the effect of the adrenergic influence on secondary lymphoid tissue would be to say that noradrenergic stimulation:

- may either enhance or inhibit the immune response - depending on the context
- augments the primary immune response
- enhances the activation or initiation of cell-mediated responses
- inhibits end-stage effector cell functions
- is essential for immunocompetence but suppresses immune function at high levels of noradrenaline
- would under basal conditions, appear to have a tonic inhibitory role which helps to curtail autoimmunity

Indications are that splenic immune responses may in turn influence the nervous system. This would be discussed in the next section as such effects are often not distinguishable form that of the circulating immunocompetent cells.

The interaction between the secondary lymphoid organs and the CNA/SAM-axis (noradrenergic activity) is summarised in Figure 4.3 (next page).

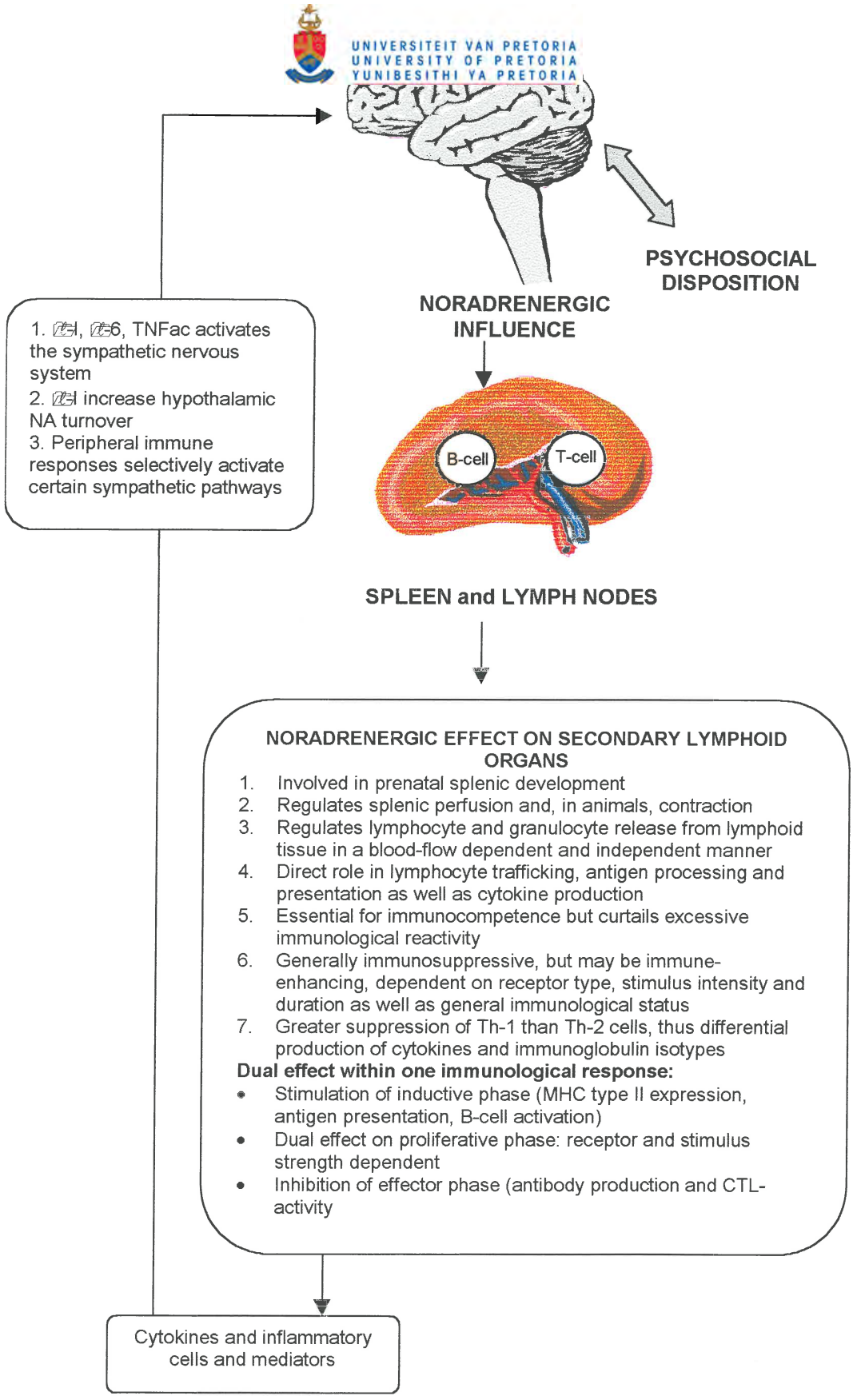


Figure 4.3: Bidirectional interaction between the secondary lymphoid organs and the CNA/SAM-axis

4.3. Bidirectional interaction between the CNA/SAM-axis, the circulating immunocompetent cells and *in vivo* immunity.

4.3.1 Effects of the CNA/SAM-axis on circulating immune cells and *in vivo* immunity.

Some of the major findings concerning the effects of sympathetic stimulation on immune cells and on *in vivo* immunological competency are referred to in Table 4.4. The following synoptic discussion is based largely on publications referred to in Table 4.4 and on references listed in Table 4.4. All references are therefore not duplicated in this discussion. The table does not include all available or only original publications as such a compilation would be beyond the scope of this writing. Because of the magnitude of the published work, references to review articles rather than original articles are sometimes provided.

Beta-adrenoceptors are found on T-and on ß-lymphocytes, macrophages, monocytes, neutrophils, basophils and on eosinophils. These receptors are upregulated in the presence of ß-blockers and down-regulated in the presence of ß-agonists, increased with cellular maturation and may increase or decrease with mitogen stimulation - depending on the signal transduction pathway [This was reviewed by Madden and Felden, 1995 (62)]. The response upon a noradrenergically induced increase in cAMP depends on the receptor density, the signal pathway, the duration of the stimulus, the time of stimulation relative to the immune stimulus, the summation of other influences and on previous immunisations (See Table 4.4 for references).

Table 4.4: The effect of sympathetic stimulation on immunological cells and on general *in vivo* immunocompetence

1. Early <u>in vitro</u> studies : a. ß-adrenoceptor stimulation (↑cAMP): Inhibition of lymphocyte proliferation, antibody secretion and production of pro-inflammatory substances b. α-adrenoceptor stimulation: Augmentation of lymphocyte proliferation, antibody secretion, production of pro-inflammatory substances	An oversimplification - complexity of interaction evidenced by following comments	Hadden et al, 1970 Melmon et al, 1974 Bourne et al, 1974 (98,99,100)
2. In general an inhibition of granulocyte function *(in vitro)*	*Inhibition of neutrophil phago-cytosis and lysozymal release *Inhibition of neutrophil respiratory burst *Decreased rate of superoxide production	Zurier et al, 1974 Nielson, 1987 Gibson-Berry et al, 1993 Yukawa et al, 1990 Rivkin et al, 1975

	*Shortened period of superoxide production (some contradiction) *Inhibition of antigen-induced histamine and SRS-A release from sensitized basophils and other cells (i.e. ↓ in IgE-mediated hypersensitivity) *A transient increase in circulating granulocytes with acute stimulation * ▼ NK cell cytotoxicity	Harvath et al, 1991 Lichtenstein and Margolis, 1968 Assem et al, 1969 Bourne et al, 1974 Ishizaka et al, 1971 Madden and Felton, 1995 (101,102,20,103, 104,105,106,107,99, 108,109,89)
3. Sympathetic stimulation can stimulate or suppress lymphocyte activity depending on the type of immunological stimulus (i.e. intracellular pathway) the duration of the stimulus and the sympathetic stimulation, the time of application of NA stimulation relative to time of stimulus (ß-cells/ß-adrenoceptor) (*in vitro*)	LPS + NA simultaneously : ↑ proliferation of spleen cells; LPS with later NA : no effect; NA ↑ proliferation after polyclonal ß-cell morigen; NA inhibits proliferation of anti IgM antibodies, differences between direct B-cell stimulation and antigen presenting cells induced stimulation	Li et al, 1990 Chartash et al, 1988 Kawakami et al, 1993 (110,111,112)
4. ß-adrenergic stimulation generally inhibits T-cell activity. Inhibition (if present) depends on ß-adrenoceptor density, duration of NA exposure and intracellular pathway (*in vitro*)	NA (and other factors which ↑cAMP)inhibits T-cell proliferation, Iℓ-2 production (↓anti-CD3-induced T-cell proliferation and enriched $CD4^+$, $CD8^+$, $CD45\ RO^+$ lymphocyte subpopulations)	Hadden et al, 1970 Kammer et al, 1988 Carlson et al, 1989 Bartik et al, 1993 (98,113,114,115)
5. Dual T-cell stimulation through ß-adrenergic and -cell receptors increases cAMP concen-tration and ▲ [CaMP] is generally associated with suppression of cell proliferation	* ▲ cAMP inhibits transmembrane signalling events which in turn prevent activated T-cells to transmit from the Go to the G1 of the cell cycle * ▲ cAMP suppresses the expression of proto-oncogenes c-myc and c-Ha-ras * ▲ cAMP in activated T-cells suppresses 1*l*-2 production	Reviewed by Roszman and Carlson, 1991 (96)
6. Lysis of cells by NK cells and Cytotoxic lymphocytes (CTL) are inhibited by ß-adrenoceptor stimulation in a dose dependent manner. Noradrenalin can influence macrophages and NK-cell activity either directly of through regulation of cytokine production *in vitro*	Lower doses (10^{-6}M) would appear to inhibited, and doses above 10^{-8}M may potentiate NK activity. CTL-induced target cell lysis is inhibited and exocytosis of granules suppressed by adrenergic stimulation	Katz et al. 1982 Hellstrand et al. 1985 Strom et al. 1973 (116,117,118)
7.a. ß-adrenoceptor activation generally inhibits stimulus- induced cytokine production (depending on timing of ß-adrenoceptor stimulation relative to immune stimulus) The reponsiveness of cells to cytokines is generally inhibited by adrenergic stimulation b. Synergistic action between	Inhibition of the production of TNF by monocytes and macrophages. IFN induced activation of macrophages is inhibited by NA. Inhibition further augmented by VIP (∴ ↑cAMP). ß-receptor stimulation +Iℓ-4 increase the expression of CD23 IgE receptors. Iℓ-2 induction of LAK cells increased by α-agonist	Severn et al, 1992 Spengler et al, 1994 Irimajiri et al, 1985 Koff and Dunegan, 1985 Beckner and Farrar, 1988 Paul-Eugene et al, 1993

adrenoceptor and cytokines may alter the response c. Isolated reports of cytokine stimula-tion by α-stimula-tion (*in vitro*)		(119,120,121,122,123,124)
8. Multicellular mechanistic Immune reponses indicate that ß-adrenergic stimulation might be essential for early events and might suppress the final events of antibody production. α-Receptors would appear to enchance the final immune reponse (*in vitro*)	NA added in beginning to cell cultures increases antibody response, NA added late in process inhibits immune response	Melmon et al, 1974 Sanders and Powell-Oliver, 1992 Ron and Sprent, 1987 (99,125,126)
9. Cytotoxic lymphocytes and antigen-induced proliferation need ß-adrenoceptor stimulation during early developmental stages. α-Stimulation may work in concert with ß-stimulation. Perhaps α and ß effects oppose each other with ß-stimulating early events and α stimulating later events with ß suppressing later stages and α earlier stages (*in vitro*)	Terbutalin and ISO at inital stages in mixed culture: ▲ CTL generation. Phentolamine: ↑lytic activity. Phenylephrine: ↓ antigen-specific proliferation of lymphoid cells	Hatfield et al, 1986 Heilig et al, 1993 Madden and Felten, 1995 (127,76,89)
10. *In vivo* experiments with chemical or surgical sympathectomy in adults indicate that sympathetic stimulation a. generally suppresses T-independent antibody responses and enhances T-dependent responses b. reduces cell mediated responses (delayed type hypersensitivity, Iℓ-2 production, CTL-activity) c. may increase of decrease cellular proliferation	a. Sympathectomy reduces antibody responses in adult rodents if 6-OHDA was used b. Neonatally sympathectomized adult aminals show enhanced antibody production - in an aged-dependent way c. Cell-mediated immune responses reduced after sympathectomy Opposite effects on T-dependentand T-independent antibody responses	Fuchs et al, 1988 Livnat et al, 1985 Hall et al, 1982 Kasahara et al, 1977 Madden and Felten, 1995 (26,128,95,129, 89)
11. Catecholamine infusion Showed that time of sympathetic stimulation relative to immune stimulus determines the immune effect. The acute effects include redistribution of immune cells and the chronic effect would indicate immune suppression (*in vivo*)	Early NA and continuous NA infusion inhibits T-cell proliferation and antibody response, but may lead to a transient increase in circulating immune cells (↑ release) - no change in blood flow	Felsner et al, 1992 Gader, 1974 Crary et al, 1983 Ernström and Sandberg, 1973 Ernström and Söder, 1975 McHale and Thornbury, 1990 (130,131,132,133,134,135)
12. The sympathetic system has a tonic inhibitory role in autoimmune disease : Observations in autoimmune and other pathologies, as well as in aging, indicate that a. the sympathetic system controls (↓) immunological reactions against "self-antigens" and in sodoing prevents auto-immune	Sympathectomy exacerbates experimental rheumatoid arthritis, hastens onset and augments inflammation and skeletal deterioration, of RA. A reduced NA activity associated with SLE, auto-immune haemolytic anaemia, and other auto-immune diseases	Levine et al, 1988 Breneman et al, 1993 Cunnick et al, 1990 Sonnenfeld et al, 1992 Dobbs et al, 1993 Madden and Felten, 1995

disease b. stress-induced enhancement of NA stimulation inhibits splenic T-cell proliferation, IFNδ production and increases vulnerability to herpes simplex and other infections agents (*in vivo*)		Rogers and Fozdar, 1996 (136,137,138,139,140,89,141)
13. Stress-induced decreases in $CD4^+$ cells, increases in NK-cell number and cytolytic activity are only significant in high sympathetic reactors to acute stress	The sympathetic system in the stress-induced modulation of the immune system is more pronounced in high sympathetic reactors to acute stress	Matthews et al, 1995 (142)
14. In auto-immune disease, sympathetic activity is reduced prior to onset of symptoms and chemical sympathectomy worsens the severity of the disease.	Evidence exists for an immune suppressor function	Madden et al, 1995 (35)
15. The immune modulatory effects of prolonged elevation of catecholamine levels differs from short term sympathetic stimulation and noradrenaline and adrenaline may have opposite immune modulating effects	Sympathetic effect depends on the duration of the stimulation and on the receptor type	Harris et al, 1995 (143)

It is, as was previously mentioned, clear that the effect of the sympathetic system on the immune system cannot summarily be considered as either inhibitory or stimulatory. The results of noradrenergic stimulation on the immune system has, for the major part, been analysed in terms of its stimulatory effect on intracellular cAMP levels. Within this context lie numerous factors that can complicate a simple deduction as to the effect of the sympathetic system on immunity. An example of this is the fact that cAMP has been reported to be essential for cellular maturation and proliferation (92) but that raised cAMP levels have also been said to suppress the expression of a number of proto-oncogenes. However, the expression of proto-oncogenes such as c-myc and c-Ha-ras are known to be important for the development of the proliferative potential of lymphocytes (96). Another confounding factor in drawing a parallel between *in vivo* sympathetic stimulation and the immune effect is the multitude of substances, other than noradrenaline, which are capable of modifying intracellular cAMP activity, some of them co-transmitters of the adrenergic system. A discussion on the control of intracellular cAMP activity is in itself a very wide subject that can easily be considered a separate independent field of research. It can, however, surely be seen as yet another connection between, or entrance for the cellular biologist into the field of psychoneuroimmunology, and by implication, into the field of psychology. A third group of confounding factors in

reading the sympathetic effect from the results of *in vivo* sympathetic stimulation is adrenoceptor distribution, expression and reactivity, but even more so the type of adrenoceptors present. Direct opposing effects have been ascribed to ß and α-receptors respectively and a variety of, especially ß-adrenoceptor subtypes, is presently being investigated.

It is therefore evident that the effect of sympathetic stimulation on *in vivo* immunoreactivity could never be interpreted as an irrevocable effect as it is intrinsically dependent on factors such as the physiological environment, the chronological order of immune vs. neurotransmitter stimulus, and the duration of the stimulus. In the light of our present knowledge and insight it is perhaps only possible to summarise the general effects in broad terms.

As such it is possible to say that it has by now unequivocally been established that the sympathetic system can, depending on the internal environmental context, exert, either a stimulatory or inhibitory influence on the immune system. It is further feasible that both stimulating and inhibiting influences may be exerted within the same immunological response. Such a potentially dual modulatory role for the adrenergic system has earlier been postulated by Madden and Livnat (92) describing

- a stimulating role during the inductive phase of the immune reponse
- either a stimulatory or inhibitory role, depending on the strength of the noradrenergic impact, during the proliferative phase
- an inhibitory role during the effector phase

In view of the complexity of predicting the effect of the noradrenergic influence on specific immune responses it would perhaps be more apt to try and define the influence on total *in vivo* immunocompetence. In such an assessment of immunological functionality one should consider the immune responsiveness to noradrenergic stimuli as well as the role in self-tolerance. A role for the adrenergic system has been described for both. The adrenergic effect on immunological responsiveness would appear to be of a dual nature, i.e., it could be immunostimulatory or immunorestraining.
An immunorestraining function for the sympathetic system is evident in (see table 4.4, as

well as reviews 35, 29 and 92 for references)

- it's reported role in prevention of autoimmune diseases
- the increased vulnerability to herpes simplex and other microbial agents during periods of excessive, stress induced sympathetic activity and
- in the enhancement of certain immune responses in the presence of sympathectomy and adrenoceptor blockers

An immunostimulating role from the noradrenergic system has also been confirmed established. The necessity of a functionally intact adrenergic system for immunocompetence is highlighted in an overview by Ackerman et al, (29) which deals with the ontogeny and senescence of the noradrenergic system's influence on immunological affecters. It is perhaps best illustrated by the similarity between the effects of ageing and that of sympathectomy. Similarities between the age-associated decline in immunocompetence and the effects of sympathetic denervation on the immune responsiveness can be seen in Table 4.5.

Table 4.5: A comparison between the effects of aging and the effects of chemical sympathectomy on the immune system.

IMMUNE RESPONSE	AGING	RESULT OF SYMPATHEC-TOMY
T-Cell responses		
Delayed-type hypersensitivity	▼	▼
Cytotoxic T-lymphocyte activity	▼	▼
con A-induced proliferation	▼	▼
Interleukin-2 production	▼	▼
Interleukin –2 receptors	▼	–
Polyclonal B-cell responses		
Lipopolysaccharide induced proliferation	▲▼	▲▼
Immunoglobulin secretion in reponse to poly-clonal B-cell stimulation	▲	▲
Antibody reponse		
Primary T-dependent antigen	▼	▼
Secondary T-dependent antigen	▲▼	▼
Primary T-independent antigen	▲▼	▲
Tumoricidal activity		
NK-cell activity	▲▼	▲
Resistance to tumor challenge	▼	▲

(Adapted from Ackerman et al, 1991 (29)) ; ▲=increased; ▼=decreased; ▲▼= increased or decreased.

More evidence in support of a role for the CNA/SAM-axis in immunocompetence was seen in the earlier subdivisions that dealt with the primary and secondary lymphoid organs, in the references in Tables 4.1,4.3 and 4.4, and in a multitude of other publications, not referred to in this writing.

It is further known that the psychological disposition of the individual plays a markedly dominant role in the immunoreactivity of the individual. This emotional influence is especially important in conditions of stress where both the two major stress axis, i.e. the HPA-axis and the CNA/SAM-axis, are stimulated. The degree to which an individual or animal feels in control of a situation would appear to be a major determinant in the outcome of this neurological influence on immunocompetence. Evidence points towards a role for the noradrenergic system in this respect (97). This is however dealt with in more detail in the chapter on the psychoimmunological interaction and in the final chapter.

In summary it can, with regard to the effect of the CNA/SAM-axis on circulating immune cells and total *in vivo* immunity, be said that

- a role for the sympathetic system in the control of the *in vivo* immune system has been shown beyond any doubt
- the magnitude on the adrenergic system on total immunity, relative to that of other influences such as other hormones and neuroactive agents, is still speculative
- the sympathetic system is necessary for immunological competence, especially during the developmental stages, but can also curb unwarranted immunological reactivity
- sympathetic activity may within the same immune response, stimulate the process (during the inductive phase) as well as suppress it (during the effector phase)
- it is feasible to accept that the outcome of high noradrenergic activity on the immune system depends on the perception of the individual. This psychological influence would, however, be deferred to a later chapter dealing primarily with this aspect.

4.3.2 Effects of circulating immune cells and substances on the CNA/SAM-axis.

The effect of the immune system on the nervous system can be assessed either through

ascertaining the alterations in the central noradrenergic system (CNA) function and structure, or by observation of the effects on peripheral adrenergic functions (SAM-axis). Direct immunological effects include (89).

- increased sympathetic firing rates in the adrenal medulla and spleen upon Iℓ-1 infusion
- increased firing rates in the ventro-medial hypothalamus, as well changes in adrenergic neurotransmitter concentrations and neuronal activity in diverse areas of the hypothalamus and brainstem upon either cytokine administration or immune stimulation
- a wide spectrum of neuroendocrine hormones which are secreted by immunological competent cells and which can either act as autocrine or paracrine regulatory hormones or can feed back to the central nervous system - especially the hypothalamus - to inhibit nervous system secretory activity. This might in turn affect central sympathetic activity
- the effects of immunologically derived cytokines that, over and above their many other central nervous effects, can modulate central noradrenergic functioning (144).

The exact cerebral targets are not always known, but peripherally derived cytokines have been implicated in a variety of psychological effects and even in some psychiatric disturbances. It was shown that such cytokines may be involved in changes in eating, sleeping and exploratory behaviour, as well as in socialisation and other behavioural functions and that they may be a factor in chronic fatigue, sickness behaviour and mood disturbances (145, 146). At present it would appear that the affects of the immune system on the central nervous system are mediated predominantly through immunologically derived cytokines and other inflammatory mediators. Various ways had been suggested by which these cytokines may reach their neural targets within the blood brain barrier (145). Cytokines are said to be transported across the blood brain barrier by cytokine-specific transport mechanisms or through areas with a high permeability for cytokines. Cytokines have further been shown to mediate their cerebral effects, either by binding to receptors on peripheral nerves, or by influencing the intracerebral prostaglandin production. There are also indications that the cytokine-producing leukocytes themselves may gain entry into the central nervous system to produce their cytokines *in situ*. Other substances said to be involved in the transduction of immunological occurrences into cerebral events include various immune modulators, as well as leukocyte-derived peptides and steroids. At least one of the

mechanisms mentioned has been shown to be involved in the immunological effect on the central noradrenergic system, i.e., the prostaglandin-dependent mechanism. In transmitting information about peripheral immunological event to the central nervous system, the immune system acts as yet another sensory system. The immune system has, in fact, in the past been likened to a sixth sense organ (147).

The effect of immunologically derived cytokines on the brain and on behaviour is the subject of Chapter 6. It is therefore necessary to summarise the influence of the sympathetic nervous system on cytokine production. Probably the most important point to be addressed is the differential effect of the catecholamines on the secretion of pro-inflammatory and anti-inflammatory cytokines. Stimulation of the β_2–adrenoceptor–cAMP-protein kinase pathway stimulates the production of anti-inflammatory cytokines such as interleukin-10 (IL-10), transforming growth factor-β (TGFβ), interleukin-4 (IL-4), interleukin-13 (IL-13) and suppresses the release of pro-inflammatory cytokines such as interferon-gamma (INFγ), interleukin-2 (IL-2) and tumour necrosis factor -alpha (TNFα). Stress-induced noradrenaline activity can therefore cause a suppression of T helper type 1 (Th1) responses and cellular immunity while stimulating T helper type 2 (Th2) responses and humoral immunity (148).

This is a very important aspect for anyone wanting to understand the effect of stress on conditions such as allergies, autoimmune diseases, chronic inflammatory conditions and probably the connection between stress-related mental disturbances and immune disturbances. However, it requires a cutting-edge knowledge of immunology and is therefore beyond the aim of this thesis. Some of the essential detail would be discussed in chapter 6 where the influence of the immune system on behaviour is dealt with.

4.4 Summary of the neuroimmunological interaction in terms of the CNA/SAM-axis.

The immune system has for long been considered an autonomous system with considerable self-regulatory capabilities. The same applies to the SAM-axis as one of the two major divisions of the autonomic nervous system. It is, however, becoming increasingly clear that the immune and sympathetic systems can be affected by other factors and that a bidirectional influence exist between them. Indications are that the bidirectional influence between the immune system and the CNA/SAM-axis can either be stimulatory or inhibitory, depending on the ontogenic stage, the

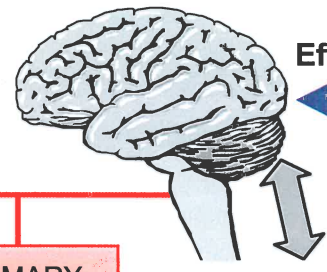
phase of the immunological reaction, the potency and duration of the stimulation as well as the magnitude of other neural and humoral influences. A sustained stimulatory bidirectional interaction would, in effect, represent a positive reverberating feedback loop between the two systems. This would appear to be the case during the early ontogenic period where sound immunological development seems to be dependent on normal functional development of the noradrenergic system, and *vice versa*. This positive functional interdependence probably exists for life - as can be seen in the effect of the immune system on receptor density and other factors, and in the positive effect of the sympathetic system during the early phases of immunological responses. However, it is evident that negative feedback between the two systems also exists, especially form the neural to the immunological system. This appears to be especially true during the effector phases of immune responses and with regard to the curtailing of unwarranted immunological activity. The CNA/SAM-axis would, in fact, appear to be a major restraining factor against the development of autoimmune disorders and in controlling certain inflammatory conditions. It would further appear that excessive noradrenergic activity, as seen during severe negative stress, could predispose to infections and tumour growth.

Recent indications of differential modulatory effects of stress-induced noradrenalin secretion on cytokine release offer extremely promising therapeutic possibilities for the treatment of stress-related disorders.

Perhaps the interactions between the two systems can best be summarised by saying that the immune system should indeed be seen as one more target of the central nervous system, and that the immune system can in turn act as an additional sensory organ informing the central nervous system about immunological related events and as such can serve as neurological, and by implication, as behavioural, response modifier.

The content of this chapter is presented on the next page, as Figure 4.4, as a heuristic diagram of the bidirectional interaction. As a small-scale version (Figure 4.1), it was also shown at the beginning of the chapter in an attempt to facilitate the reading of the chapter. It is given here in normal size as a summary of the work presented and because it is considerably easier to read.

NORADRENERGIC ACTIVITY and IMMUNITY



PHYCHOSOCIAL DISPOSITION

Effects of the immune system on the noradrenergic activity

THYMIC EFFECT ON NORADRENERGIC FUNCTION AND NORADRENERGIC CONTROL CENTRES

1. Necessary for development of neuro-secretory function during early development
2. Maintenance of adrenoceptor density
3. Regulation of adrenoreceptor responsiveness
4. Neuroregulatory effects through thymus-derived cytokines (IL-1, IL-3, MCSF, etc.), thymus-derived hormones (prolactin, ADH, oxytocin, tachykinins etc.) and thymic peptides
5. Behaviour effects include increase in spontaneous behaviour, suppression of anxiety and increased resistance to stress

NORADRENERGIC EFFECTS ON THYMUS

1. Regulatory function in thymus perfusion and release of cells from thymus
2. Necessary for early post-natal development, including thymocyte differentiation and receptor expression
3. Controls thymic micro-environment by influencing intra-thymic neurohormonal and peptidergic secretion
4. Essential for thymus-dependent immunological competence
5. Involved in thymus involution
6. Controls stimulus responsiveness of cells
7. Controls excessive immunological responses
8. May be a factor in gender-dependent differences in immunological responsitivity through interaction with steroid hormones

Effects on immune system

IN VIVO IMMUNO - REACTIVITY

SECONDARY LYMPH ORGANS

PRIMARY LYMPH ORGANS

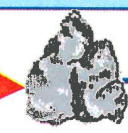
NORADRENERGIC EFFECT ON SECONDARY LYMPHOID ORGANS (SPLEEN)

1. Involved in prenatal splenic development
2. Regulates splenic perfusion
3. Regulates lymphocyte and granulocyte release from lymphoid tissue in a blood-flow dependent and independent manner
4. Direct role in lymphocyte trafficking, antigen processing and presentation as well as cytokines production
5. Essential for immunocompetence but curtails excessive immunological reactivity
6. Generally immunosuppressive, but may be immune-enhancing, dependent on receptor type, stimulus intensity and duration as well as general immunological status
7. Dual effect within same immunological reaction with stimulation in the inductive phase and inhibition in the effector phase
8. B_2-adrenoceptor-cAMP-protein kinase activity inhibits pro-inflammatory pathway and stimulates anti-inflammatory pathway through differential effects on TH1 and TH2 cells. Can shift immune response from cellular to humoral

NORADRENERGIC EFFECT ON BONE MARROW

Role in perfusion, cellular migration and leukocyte release

BONE MARROW

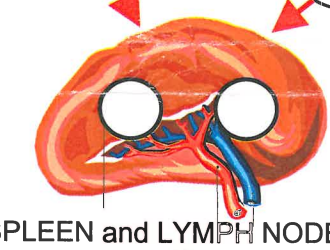


THYMUS

- Thymosins
- Thymopeptins
- Thymulin
- Cytokines
- Tachykinins
- Neurohypophysial hormones

B-cells

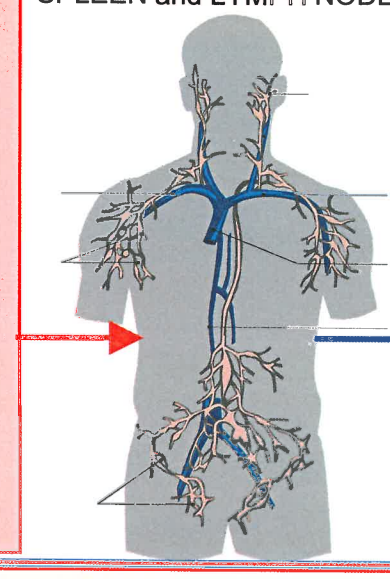
T-cells



SPLEEN and LYMPH NODES

NORADRENERGIC EFFECT ON IN VIVO IMMUNOREACTIVITY

1. Beta-adrenoceptors are found on lymphocytes, monocytes granulocytes and macrophage
2. Alpha-adrenoceptors are found on some immune cells and on vasculature (important in cell traffic and release)
3. Most phagocyte functions appear to be suppressed by NA
4. Essential for T-cell response (cytotoxic T-cell activity, Con A-induced proliferation, IL -2 production, IL -2 receptor expression, T-cell dependent antibody response) but control excessive reactivity
5. Tonic inhibition of tumoricidal activity (NK-cell activity etc) and T-independent antibody responses (polyclonal B-cell responses)
6. Inhibition of macrophage antigen presenting activity
7. Immunological effects of NA stimulation are dependent on receptor type and density, intensity and duration of stimulation as well as on stage of immunological reaction and immunology status
8. Effects on immune responses influence cytokine production
9. Helps to localize inflammatory response
10. Protects body from the tissue damage inflicted by overproduction of systemic proinflammatory cytokines
11. Stress-induced adrenergic stimulation may expose the body to infectious complications and autoimmune diseases and tumour growth
12. Hypoactivity may predispose to fibromyalgia/chronic fatigue syndrome



EFFECTS OF THE IMMUNE SYSTEM ON NEUROLOGICAL FUNCTION.

1. Sensory function
2. Affects neuronal firing rate
3. Affects rate of noradrenaline secretion and stimulates adrenoceptor expression
4. Regulatory feedback through immunocompetent cell-derived cytokines, neuro-endocrine and peptidergic substances
5. Possible antibody – induced cerebral effect
6. Stimulation of the central stress response with a basal tonic inhibitory effect on stress-related behaviour

SUMMARY OF NEUROIMMUNOLOGICAL INTERACTION EFFECTS OF NORADRENERGEC ACTIVITY ON IMMUNITY

1. Necessary for immunodevelopment and immunocompetence
2. Major factor in immunorestraint
3. Essential for self-tolerance (basal tonic inhibitory effect helps to curtail auto-immune reactivity)
4. Opposite effects with acute (enhancement) and chronic (suppression) hyperstimulation
5. Variations in noradrenergic activity implicated in tumourgenesis, chronic inflammatory disorders, stress-induced Immuno-incompetence and allergies
6. Implicated as a factor in the immune alterations obtained through conditioning and in immune alterations with drug abuse

EFFECTS OF THE IMMUNE SYSTEM ON NORADRENERGIC ACTIVITY

1. Necessary for neurosecretory development and maintenance of adrenoceptor expression and functional integrity
2. Acts as sensory system to alert CNS about peripheral infectious and immunological occurences
3. Immunologically-derived cytokines give rise to the central stress response through induction of stress hormone secretion
4. (HPA-axis and CNA/SAM-axis) and inhibition of anti-stress hormones (GH, LH, FSH etc)
5. Immunologically-derived cytokines implicated in behavioural effects such as sickness behaviour, mood changes, sleep, eat and locomotor disturbances, anxiety, as well as exploratory, coping and related behavioural phenomena
6. Excessive immunological inflammatory activity may predispose to psychological disorders
7. Cytokine therapy can have neurotoxic effects partially mediated via central NA system

Figure 4.4: A schematic representation of the bidirectional interaction between the CNA/SAM-axis and the immune system.

Legend to Figure 4.4

Figure 4.4: A schematic representation of the bidirectional interaction between the CNA/SAM-axis and the immune system

[On the left hand side of the diagram the effects of the CNA/SAM-axis on primary lymphoid organ (thymus and bone marrow) immunity, secondary lymphoid organ (spleen and lymph nodes) immunity, and on *in vivo* immunological reactivity and competence are shown. The right hand side represents the effects of the immune system on the functional and developmental aspects of the CNA/SAM-axis. At the bottom of the page the overall effects are summarised.]

This chapter presented the neuroimmunological interaction between the CNA/SAM-axis and the immune system. In combination with the previous chapter, i.e., Chapter 3, it describes psychoneuroimmunology in terms of the CNA/SAM-axis. The next chapter will show the psychoneuroimmunological interaction in terms of the CRH/HPA-axis.

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