Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 82, Suppl. II: 1-8, 1987.
Intern. Symp. on Immunomodulators

THYMULIN : BIOCHEMISTRY, BIOLOGY AND THERAPEUTICAL APPLICATIONS

MIREILLE DARDENNE & J.F. BACH

INSERM U 25 - Hôpital Necker - 161, rue de Sèvres 75730, Paris Cedex 15, France

Thymulin is a pharmacologically active metallononapeptide inducing the differentiation of T cells and enhancing several functions of the various T cell subsets in normal or partially thymus-deficient recipients. Its effect on suppressor T cells is, so far, the most remarkable and should be the first to find useful clinical applications. The peptide is a natural hormone, available in synthetic form. It is not toxic and one may foresee its clinical use as one of the major immunoregulatory agents in the near future.

The thymus produces a number of polypeptides. Several of them are pharmacologically active on lymphoid cells and have been considered as putative thymic hormones (Bach, 1983; Goldstein et al., 1987).

One should, in fact, be more restrictive and reserve the name of thymic hormones to substances that have been fully characterized chemically and biologically and whose secretion by the thymus shows the features of a true hormone. In other words, one should only consider as a thymic hormone chemically purified substances (preferably sequenced and synthesized in case of peptides), exclusively secreted by the thymic epithelium and acting upon lymphoid cells after binding to specific receptors. Only three peptides fulfill all these conditions: Thymopoietin (Goldstein et al., 1987), Thymosin α1 (Goldstein et al., 1983), and thymulin, formerly called facteur thymique sérique (FTS), matter of this review (Bach, 1983). Importantly, a number of arguments indicate that these peptides represent major (although not necessarily exclusive) signals playing a crucial role in intrathymic T cell differentiation.

From the pharmacological view point, thymic hormones can be considered as promising potential immunomodulating agents. By their diversified actions on T cells, they can stimulate the various T cell subset functions. This multiplicity of action may be beneficial since it widens their potential therapeutic indications but, conversely, may pose a difficult problem when the action on suppressor T cells opposes that on helper or effector T cells. The immunopharmacology of thymulin will be discussed in terms of in vitro and in vivo effects (in experimental animals and in humans), kinetics and toxicity. We shall also present the preliminary clinical trials in rheumatoid arthritis.

BIOCHEMISTRY

A. Chemical analysis – Thymulin is a metallononapeptide coupled to zinc with the following amino acid sequence: P-Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn (Bach et al., 1977).

Recent data using immunoblotting analysis with anti thymulin monoclonal antibodies indicate that the hormones is produced in the epithelial cells as a pro-hormone of higher molecular weight (Savino et al., 1985). DNA recombinant studies in progress will reveal the amino-acid sequence of the said precursor.

The aminoacid sequence has been established on porcine serum derived hormone (Dardenne et al., 1977). Aminoacid analysis of the peptide present in calf thymic extract (Dardenne et al., 1980) and human plasma shows an identical aminoacid composition. Thus, likely the amino-acid sequence is identical in mice, calves and humans.

B. Synthesis - Zinc dependency – The peptide has been synthesized by a number of laboratories using either solid phase or liquid phase synthesis (Bricas et al., 1977; Strachan et al., 1979; Lefrancier et al., 1984). The synthetic material shows identical activity the natural hormone, provided zinc is added in adequate amounts (mole to mole ratio) (Dardenne et al., 1982). The absence of optimal zinc complementation may lead to less active or inactive preparations as may be those in which the metal is that commonly in the synthesis reagents without deliberate concentration adjustment. Optimal zinc binding needs previous peptide passage on a chelating agent, probably to remove other metals which could occupy the zinc binding site. Zinc-thymulin relationship has been determined by several biochemical and physical studies. The binding is stoichiometic

and is optimal at pH 7. Its affinity, studied by equilibrium chromatography, is 10^{-7} M. Lastly, nuclear magnetic resonance studies have shown that zinc induces a tetrahedric conformation with direct binding to Asn and the two Ser residues (Laussac et al., 1985). It is interesting to note that the two peptide conformations, the zinc coupled biologically active one and the zinc-free inactive one, show distinct antigenic determinants which can be distinguished by monoclonal antibodies (Dardenne et al., 1985).

C. Structure-function relationships – The active site of the molecule has been studied by the functional analysis of more than 50 hormone analogs synthesized by E. Bricas and collaborators (Blanot et al., 1979; Martinez et al., 1980). The first two N-terminal residues (p-Glu and Ala) are not necessary for the biological activity but the second one Ala contributes to it since the heptapeptide only shows partial biological activity. Receptor studies to be described below indicate that the receptor binding site comprises residues 5-7, and that the triggering site, necessary for the biological activity, includes residues 8-9. It interesting that some biologically inactive analogs, notably those bearing aminoacid substitutions on the C-terminal residues bind to the receptor but show antagonistic effect, as assessed in the rosette assay (Pléau et al., 1979). Note also that long-lived analogs have been synthesized but that these compounds have not been submitted to extensive pharmacological evaluation since the original peptides is fully active in vivo when coupled to zinc; zinc coupling simultaneously induces the biologically active conformation and increases the life span of the peptide (Dardenne et al., 1982).

PHYSIOLOGY

A. Secretion – Thymulin is physiologically produced by the thymic epithelium. This is its exclusive origin since thymectomy induces the complete disappearance of the hormone from the circulation (Bach & Dardenne, 1973) and monoclonal antibodies produced against the hormone selectively bind to the thymic epithelium and not to other tissues (Savino et al., 1982). This is a remarkable feature of thymulin since other putative thymic hormones, such as thymosins, are produced by other organs. Thymulin production by the thymus gland is submitted to a fine feed-back regulation, as illustrated by the increase of the number of thymulin-containing cells in the thymus in vivo after peripheral depletion of the hormone induced by passive or active immunization (Savino et al., 1983) or by in vitro culture of thymic explants free of the in vivo inhibitory signals (Cohen et al., 1986). This in vitro system has indicated that the inhibitory signal might be the hormone itself since thymulin addition to the culture decreases the number of detectable thymulin-containing cells (Cohen et al., 1986).

B. Age-dependency – Thymulin synthesis declines with age and no more hormone is detected in the circulation after the age of 6 months in mice (Bach & Dardenne, 1973) and 60 years in humans (Bach et al., 1972). The hormone synthesis is stimulated by thyroid hormones (Savino et al., 1984) steroids (Savino et al., in press), and unexpectedly cyclosporine (Dardenne et al., 1987). It is depressed, but only at very high doses, by corticosteroid.

IMMUNOPHARMACOLOGY

A. In vitro effects – We first described in 1971 that thymic extracts were able to induce T cell markers in vitro in immature lymphoid cells that were deprived of such markers (Bach et al., 1971). The assay that was initially used was based on the induction of the sensitivity to inhibition by anti-Thy-1 serum and azathioprine of rosette formation with sheep erythrocytes by a minor subpopulation of bone marrow cells from normal mice or of spleen cells from adult thymectomized mice. Ultimately, these data were confirmed by using monoclonal anti-Thy-1 antibodies and cytofluorograph cell analysis. Thymulin has been shown to induce most T cells markers, both in mice and humans (Incefy et al., 1980; Abiko et al., 1979; Bene et al., 1982) (Table 1). It is not yet clear, however, whether the peptide induces de novo synthesis of the antigen (gene activation) or enhances its expression (membrane rearrangement). The effects on Rosette Forming Cells (RFC) are particularly interesting since they involve a unique minor T cell subset (Lyt 1^-, L3T4$^-$, Lyt2^+) expressing antigen-specific T cell receptors (our unpublished observations). Thymulin does not modulate the expression of the receptor itself but that of molecules associated with the receptor itself but that of molecules associated with tor itself (such as the Lyt 2 antigen). It should be made clear, however, that these in vitro effects do not suffice to assess the differentiating capacities of the hormone since a number of other materials, notably those in-

TABLE I
Effects of thymulin on membrane markers

	In vitro	In vivo
Sheep erythrocyte rosettes		
Mouse		
Sensitivity to azathioprine, anti-Try-1 and xenogenic antilymphocyte antibodies	Normal bone marrow cells Adult thymectomized or nude mice	Adult thymectomized mice
Number of thymus rosette-forming cells	Normal mice (thymus)	Normal mice (thymus)
Human		
Total E rosettes	Immunodeficiency PBL[a] and bone marrow cells	Immunodeficiency diseases
Active E rosettes	Uraemic patients	
Theophilline-treated E rosettes	Normal PBL	
Autologous rosette forming cells		
Mouse	Adult thymectomized and nude mice	Adult thymectomized mice Normal mice (thymus)
Human	Normal PBL	
Differentiation antigens		
Mouse		
Thy-1	BSA-separated normal mouse spleen cells	Nude mice
Lyt 1,2,3	Adult thymectomized mice	
Human		
Xenogeneic antisera-defined antigens	Normal bone marrow cells	
OKT-and Leu-defined antigens	Ataxia telangiectasia	Immunodeficiency diseases
Other receptors		
PNA binding (decrease)	Normal bone marrow cells	
FCγ-binding T cells	Lupus patients	

aPBL: peripheral blood lymphocytes

creasing intra-cellular cyclic AMP levels, induce non specifically the same effects.

B. In vivo effects – Thymulin has also been shown to induce meaningful in vivo effects on various T cell functions (Table II). As already mentioned above, this diversity of action may represent a difficulty in assessing the hormone properties since cells with opposed functions (namely helper and suppressor) may be simultaneously stimulated. It is also important to stress that the effect varies with the recipient, the function tested and the dosage. Adult thymectomized mice are the most sensitive to the effect of the hormone. After in vivo treatment whith small amounts of the peptide (1-100 ng), they recover a normal differentiation antigen phenotype (Dardenne et al., 1980), lose their increased expression of self receptor for autologous red cells (Charreire & Bach, 1975), normalize their decreased capacity to proliferative response in the presence of PHA (Kaiserlian & Dardenne, 1982) or to generate cytotoxic T cells (Bach, 1977). Similarly, NZB mice which, like adult thymectomized mice, show a premature decline of thymulin production (Bach et al., 1973), are highly sensitive to in vivo treatment with the hormone which decrease the abnormally high production of antibodies against polyvinylpyrrolidone (Bach & Niaudet, 1976) or autoantibodies (e.g. anti-erythrocytic) (Israel-Biet et al., 1983). Less clear results are obtained in totally T cell-deprived nude mice, which only recover expression of differentiation alloantigens without function. In normal mice with normal thymulin production the only effect noted deals with suppressor T cells whose function can be exacerbated after injection of relatively high doses of the peptide (1-10μg) (Kaiserlian et al., 1981). In particular, one may depress delayed type hypersensivity reactions to sheep red blood cells or dinitrofluorobenzone (Erard et al., 1979), antibody production against

TABLE II

Effects of thymulin on T cell functions

Proliferation	
PHA-induced	Mouse, mice Tx at three weeks
	Rat, ATx rats
	Human, immunodeficiencies
Autologous mixed lymphocyte reaction (lupus)	
Cytotoxicity and delayed hypersensitivity	
Allogeneic cytotoxicity	ATx mice
Anti-TNP cytotoxicity	Normal thymocytes
Graft-Versus-Host reaction	Normal mice
Rejection of MSV-induced sarcoma (low dose)	B mice
Stimulation of delayed-type hypersensitivity	ATx mice
Helper T cells	
Antibody production (SRBC)	Ageing mice
Induction of IgA (and IgE)	Ataxia telangiectasia and variable immunodeficiency
Production of interleukin-2	Normal thymocytes and nude mouse spleen cells
Increase in anti-DNA IgG autoantibodies	Young B/W mice (females)
Suppressor T cells	
Retardation of skin allograft rejection	Normal mice
Depression of antibody production	
SRBC	Normal mice
PVP	NZB mice
DNA	B/W mice (males)
Depression of T cell mediates cytotoxicity	Normal mice
Depression of delayed-type hypersensitivity	Normal mice
Stimulation of Con A-induced suppression	Lupus patients
	Normal subjects.

sheep red blood cells, or generation of cytotoxic T cells against allogeneic target cells (Bach, 1977).

Finally, these data suggest that thymulin may influence the whole T cell lineage from the pre-cells, as present in nude mice, to the mature T cells found in normal mice including both helper and suppressor subsets. All these cells probably express thymulin receptors whose stimulation may lead to increased or even de novo expression of surface antigens and eventually, but not necessarily, functional differentiation. The whole maturation process probably needs the simultaneous effect of other signals, notably presentation of major histocompatibility complex products. Note also that mature T cell only express some function sensitivity at relatively high pharmacological doses of the hormone, with a preferential effect on the suppressor subset.

C. Cellular mode of action – thymulin binds to specific receptors whose presence has been demonstrated on T cell derived lymphoblastoid lines (Pléau et al., 1980; Gastinel et al., 1982). Such receptors present two sites of high (10^{-9} M) and low (10^{-7} M) affinity. Receptor binding is inhibited by thymulin agonists and antagonists but shows no reactivity with inactive analogues. Interestingly, the zinc-deprived hormone binds to the receptor (and behaves as an antagonist) but lacks the triggering site (Pléau et al., 1979). Prostaglandin E_2 (PGE_2) could represent the second messenger since thymulin induces in vitro PGE_2 synthesis both in mouse thymocytes (Rinaldi-Garaci et al., 1985) and in human peripheral blood lymphocytes (Gualde et al., 1982). In addition, PGE_2 mimicks some of thymulin properties, notably its action on RFC (Bach & Bach, 1973) and indomethacin, an inhibitor of prostaglandin synthetase, blocks the capacity of Thymulin to induce T cell markers in immature T cell (Bach & Charreire, 1979). One cannot exclude, however, that cyclic AMP is not the biologically significant second messenger since PGE_2 stimulates cyclic AMP synthesis by lymphoid cells and cAMP also mimicks the effects of thymulin on its target cells and even synergizes with it (Bach & Bach, 1973).

PHARMACOKINETICS AND TOXICITY

The *pharmacokinetics* of thymulin have been studied in detail by use of the rosette assay which permits quantitative evaluation of circulating thymulin levels. The half-life of the zinc coupled peptide is of the order of 30 min. Several long-lived analogues have been synthesized but these analogues have not yet been used clinically since the native compound can successfully induce T cell functions on its own, when injected once daily or once every other day. Pharmacokinetic studies have indicated that the stoeichiometric ratio 1 : 1 of the zinc peptide was optimal for in vivo peak and half life (Dardenne et al., 1982). The problen of kinetics is complicated by the existence of several molecules behaving like *carriers* and/or inhibitors. The presence of carriers in mouse and human serum is indicated by the biological activity of serum fractions (separated by G-25 Sephadex chromatography) at molecular weight elution close to that of albumin (Dardenne et al., 1980). This activity is not due to an intrinsic property of albumin, prealbumin or a similar molecule, as was once thought (Burton et al., 1978), since the activity of the albumin fraction is not seen in thymectomized mouse and can by induced in those mice injecting synthetic thymulin.

There are also thymulin *inhobitors* which are at least parly distinct from carriers. Some of these inhibitors seem to act on the same targets as thymulin without direct interaction whith the peptide since they can readily be separated from thymulin by mere filtration on an Amicon membrane. Their thymus-dependency is suggested by their absence in thymectomized mice. They could be 1/ large or small molecular weight thymulin inactive analogues (precursors or split products); 2/ protein bound thymulin or 3/ any substance produced by the thymus or under its influence. Other inhibitors are probably anti-thymulin autoantibodies as demonstrated in autoimmune (NZB x NZW)F_1 and db/db mice (Dardenne et al., 1984). The presence of all these carriers and inhibitors probably influences the evaluation of circulating thymulin level. They might also explain, in addition to the zinc dependency of the hormone antigenicity, the difficulties met in establishing a satisfatory radioimmunoassay (the only assay presently available using rabbit anti-thymulin serum (Pléau et al., 1977) does not detect the circulating hormone).

Combining immunopharmacological and pharmacokinetics data, we have proposed to use thymulin at *two dose ranges* according to the desired effect. In mice, helper and effector T cells are stimulated by doses of 1-100 ng and suppressor T cells at doses of 1-10 μg (Kaiserlian et al., 1981). In human, the two equivalent dose ranges are 50-500 μg (helper/effector T cells) and 1-10mg (suppressor T cells). More work is necessary, however, to document further such dose choice.

The *toxicology* of thymulin has been studied in several species. No signs of toxicity were found whatever with doses higher than 100 times those just mentioned above, for treatment periods ranging from 1 to weeks.

PRELIMINARY CLINICAL TRIALS

The clinical use of thymulin is based on several elements. The existence of a definite thymic deficiency represents the best argument especially when it is substantiated by a reduced circulating thymulin level. This is the case of the Di George syndrome (Iwata et al., 1981) or of ataxia-telangectasia (Bordigoni et al., 1982) and to a lesser degree, of some forms of systemic lupus (Bach, 1983). In other settings, there may not be any obvious thymic deficiency but the hormone has been shown to possess potentially useful pharmacological properties. It is interesting, in that regard, that thymulin improves some parameters of the autoimmune disease of NZB and (NZB x NZW) F_1 mice (Bach, 1983; Israel-Biet et al., 1983) and prevents the onset of experimental allergic encephalomyelitis in the guinea pig (Nagai et al., 1982) and experimental allergic thyroiditis in the mouse (Tomazic & Elias, 1985).

Two main clinical indications have been the matter of preliminary clinical trials. Immunodeficient children with Di George syndrome, ataxiatelangectasia and common variable immunodeficiency have been treated successfully with thymulin at low dosage (50-250 μg) (Bordigoni et al., 1982). A significant effect was observed both on immunological parameters and clinical symptoms. Patients with rheumatoid arthritis have also been treated with thymulin at higher doses (1 to 10mg/day) for long periods of time (up to 6 months). Two open trials and two randomized trials were performed (Amor et al., 1984; 1987). A significant improvement was observed at higher doses (notably 5 mg) in the double blind randomized

trial on several parameters such as grip strength, morning stiffness, pain and overall subjective appreciation of the patient. Interestingly, this effect was not clearly associated with an improvement of the immunological abnormalities often noted in these patients, nor with a clear reduction of inflammatory signs (Amor et al., 1987). Note that, as other immunostimulants, tymulin was inactive in AIDS when given at the late stage of the disease.

Other indications could be envioned in the near future. As effector T cell stimulant, thymulin could be tried in recurrent herpes and fungal infections (preliminary data in mice and humans were encouraging). As suppressor T cell stimulant, it could be used in systemic lupus. Sjögren's syndrome (which is dramatically improved in the mouse) (Bach et al., 1979) and other autoimmune diseases such as type I diabetes (in a second stage, once the disease has been stopped by immunosuppression) or in atopic diseases (asthma or atypic dermatitis).

REFERENCES

ABIKO, T.; KUMIKAWA, M. & DAZAI, S., 1979. The effect of STF and its analogs on T cell with cellular immunodeficiency. *Chem. Pharm. Bull., 27* :2207-2211.

AMOR, B.; DOUGADOS, M.; MERY, C.; DARDENNE, M. & BACH J.F., 1987. Nonathymulin in rheumatoid arthritis: two double blind, placebo controlled trials. *Ann. Rheumatic Diseases, 46* :549-554.

AMOR, B.; DOUGADOS, M.; MERY, C.; de GERY, A.; CHOAY, J.; DARDENNE, M. & BACH, J.F., 1984. Thymuline (FTS) in rheumatoid arthritis. *Arthritis Rheum., 27* :117-118.

BACH, J.F., 1983. Thymulin (FTS-Zn). *Clin. Immunol. Allergy, 3* :133-157.

BACH, J.F., 1983. Thymic factors and immunoregulation role in autoimmune diseases p. 20-29. In *Regulation of the Immune response.* (D.M. Jacobs, P.L. Ogra, Eds.) Karger Basel.

BACH, J.F. & DARDENNE, M., 1972. Thymus dependency of rosette forming cells. Evidence for a circulating thymic hormone. *Transplant. Proc., 4* :345-349.

BACH, J.F. & DARDENNE, M., 1973. Studies on thymus products. II. Demonstration and characterization of a circulating thymic hormone. *Immunology, 25* :353-366.

BACH, J.F.; DARDENNE, M.; GOLDSTEIN, A.; GOLDSTEIN, A.; GUHA, A., & WHITE, A., 1971. Appearance of T-cell markers in bone marrow rosette forming cells after incubation with purified thymosin, a thymic hormone. *Proc. Natl. Acad. Sci. USA, 68* :2734-2738.

BACH, J.F.; DARDENNE, M.; PAPIERNIK, M.; BAROIS, A.; LEVASSEUR, P. & LE BRIGAND, H., 1972. Evidence of a serum factor produced by the human thymus. *Lancet, i* :1056.

BACH, J.F., DARDENNE, M., PLEAU, J.M. & ROSA J., 1977. Biochemical characterization of a serum thymic factor. *Nature, 266* :55-56.

BACH, J.F., DARDENNE, M. & SALOMON, J.C., 1973. Studies on thymus products. IV. Absence of serum "thymic activity" in adult NZB and (NZB x NZW) F1 mice. *Clin. Exp. Immunol., 14* :247-256.

BACH, J.F. & NIAUDET, P., 1976. Thymic function in NZB mice. II. Regularly influence of a circulating thymic factor on antibody production against polyvinylpyrolidone in NZB mice. *J. Immunol., 117* :760-764.

BACH, M.A., 1977. Lymphocyte-mediated cytotoxicity: effects on ageing of adult thymectomy and thymic factor. *J. Immunl., 119* :641-648.

BACH, M.A. & BACH, J.F., 1973, Studies on thymus products. IV. The effects of cyclic nucleotides and prostaglandin on rosette forming cells. Interactions with thymic factor. *Eur. J. Immunol., 3* :778-793.

BACH, M.A. & CHARREIRE, J., 1979. Role of a circulating thymic factor in self recognition and self tolerance. *Ann. N.Y. Acad. Sci., 332* :55-63.

BACH, M.A.; FOURNIER, C. & BACH J.F., 1979. Biological activities and site of action of the circulating thymic factor. p. 177-188. In *Proc. 12th Leukocyte Culture Conference* (M. Quastel, Ed.) Acad. Press. New York.

BENE, M.C.; FAURE, G.; BORDIGONI, P.; OLIVED. & DUHEILLE J., 1982. In vitro induction of monoclonal antibody defined T cells markers in lymphocytes from immune deficient children by synthetic thymic factor (FTS). *Clin. Exp. Immunol., 48* :423-428.

BLANOT, D.; MARTINEZ, J.; AUGER, G. & BRICAS, E., 1979. Synthesis of analogs of the serum thymic monapeptide 'facteur thymique sérique' (FTS) (part I). *Intern. J. Peptide Protein Research., 14* :41-56.

BORDIGONI, P.; BENE, M.C.; BACH, J.F.; FAURE, G.; DARDENNE, M.; DUHEILLE, J. & OLIVE, D., 1982. Improvement of cellular immunity and IgA production in immunodeficient children after treatment with synthetic serum thymic factor (FTS). *Lancet, ii* :293-297.

BRICAS, E.; MARTINEZ, T.; BLANOT, D.; AUGER, M.; DARDENNE, M.; PLEAU, J.M. & BACH, J.F., 1977. The serum thymic factor and its synthesis, p. 564-567. In: Goodman, M. & J. Meienhofer Eds. Proc. 5th Intern. Peptide Symp. John Willey, New York.

BURTON, P.; IDEN, S.; MITCHELL, K. & WHITE, A., 1978. Thymic hormone-like restoration by human prealbumin of azathioprine sensitivity of spleen cells from the thymectomized mice. *Proc. Natl. Acad. Sci. USA, 75* :823-827.

CHARREIRE, J. & BACH, J.F., 1975. Binding of autologous erythrocytes to immature T cells. *Proc. Natl. Acad. Sci. USA, 72* :3201-3205.

COHEN, S.; BERRIH, S.; DARDENNE, M. & BACH, J.F., 1986. Feed-back regulation of the secretion of a thymic hormone (thymulin) by human thymic epithelial cells in culture. *Thymus, 8* :109-119.

DARDENNE, M.; PLEAU, J.M. & BACH, J.F., 1980. Evidence for the presence of a carrier of a serum thymic factor (FTS). *Eur. J. Immunol., 10* :83-86.

DARDENNE, M.; PLEAU, J.M.; BLOUQUIT, J.Y. & BACH, J.F., 1980. Characterization of facteur

thymique sérique (FTS) in the thymus. II. Direct demonstration of the presence of FTS in thymosin fraction V. *Clin. Exp. Immunol., 42* :477-482.

DARDENNE, M.; PLEAU, J.M.; MAN, N.K. & BACH, J.F., 1977. Structural study of circulating thymic factor : a peptide isolated from pig serum. I. Isolation and purification. *J. Biol. Chem., 252* :8040-8044.

DARDENNE, M.; NABARRA, B.; LEFRANCIER, P.; DERRIEN M.; CHOAY, J. & BACH, J.F., 1982. Contribution of zinc and other metals to the biological activity of the serum thymic factor (FTS). *Proc. Natl. Acad. Sci. USA, 79* :5370-5375.

DARDENNE, M.; SAVINO, W. & BACH, J.F., 1984. Autoimmune mice develop antibodies to thymic hormones. Obtention of anti-thymulin monoclonal antibodies from diabetic (db/db) and B/W mice. *J. Immunol., 133* :740-743.

DARDENNE, M.; SAVINO, W.; BERRIH,S. & BACH, J.F., 1985. Evidence for a zinc dependent antibody binding site on the molecule of thymulin, a thymic hormone. *Proc. Natl. Acad. Sci., 82* :7035-7038.

DARDENNE, M.; SAVINO, W.; FEUTREN, G. & BACH J.F., 1987. Stimulatory effects of CyA on mouse and human thymic epithelial cells. *Eur. J. Immunol., 17* :275-279.

ERARD, D.; CHARREIRE, J.; AUFREDOU, M.T. & BACH, J.F., 1979. Regulation of contact sensitivity to DNFB in the mouse : effects of ageing on adult thymectomy and thymic factor. *J. Immunol., 123* :1573-1576.

GASTINEL, L.N.; DARDENNE, M.; PLEAU J. M. & BACH J.F., 1984. Studies on the zinc binding sites to the serum thymic factor thymulin. *Biochem. Biophys, Acta, 797* :147-155.

GASTINEL, L.N.; PLEAU, J.M.; DARDENNE, M.; SASAKI, A.; BRICAS, E.; MORGAT, J.K. & BACH J.F., 1982. High affinity binding sites on plasma membranes obtained from the lymphoblastoid cultured 1301 cell line for highly radioactive serum thymic factor. *Biochem. Biophys. Acta, 684* :117-126.

GOLDSTEIN, G.; BACH, J.F. & WIGZELL, H. (Ed) 1987. Overview of immunoregulation by thymopoietin. UCLA symposia on molecular and cellular biology, p. 51-59. New Series. Vol. 41. Alan R. Liss, Inc. New York, N.Y.

GOLDSTEIN, A.L.; LOW, T.L.K.; ZATZ, M.M.; HALL, N.R. & NAYLOR, P.H., 1983. Thymosins. *Clin. Immunol. Allergy* (London), *3* :119-132.

GUALDE, N.; RIGAUD, M. & BACH, J.F., 1982. Stimulation of prostaglandin synthesis by the serum factor (FTS). *Cell. Immunol., 70* :362-366.

IMAIZUMI, A.; GYOTOKU, J.; TERADA, S. & KIMOTO, E., 1981. Structural requirement for the biological activity of serum thymic factor. FEBS *Letters, 128* :108-110.

INCEFY, G.S.; L'ESPERANCE, P. & GOOD, R.A., 1975. In vitro differentiation of human marrow cells into T lymphocytes bu thymic extracts using the rosette technique. *Clin. Exp. Immunol., 19* :475-483.

INCEFY, G.S.; MERSTELSMANN, R.; YATA, K.; DARDENNE, M.; BACH, J.F. & GOOD, R.A., 1980. Induction of differentiation in human marrow T cell precursors by the synthetic serum thymic factor (FTS). *Clin. Exp. Immunol., 40* :396-400.

ISRAEL-BIET, D.; NOEL, L.H.; BACH, M.A.; DARDENNE, M. & BACH, J.F., 1983. Reversal of the autoimmune disease of (NZB x NZW) F1 mice by cyclosporin A and thymulin (FTS-Zn). *Clin. Exp. Immunol., 54* :359-?.

IWATA, T.; INCEFY, G.S.; CUNNINGHAM-RUNDLES, S.; CUNNINGHAM-RUNDLES C.; SMITHWICK, E.; GELLER, N.; O'REILLY, R., & GOOD, R.A., 1981. Circulating thymic hormone activity in patients with primary and secondary immunodeficiency disease. *am. J., Med., 71* :385-394.

KAISERLIAN, D. & DARDENNE, M., 1982. Studies on the mechanisms of the inhibitory effects of serum thymic factor on murine allograft immunity. *Cell. Immunol., 66* :360-371.

KAISERLIAN, D.; DUJIC, A.; DARDENNE, M.; BACH, J.F.; BLANOT, D. & BRICAS, E., 1981. Prolongation of murine skin grafts by FTS and its synthetic analogues. *Clin. Exp. Immunol., 45* :338-342.

LAUSSAC, J.P.; HARAN, R.; DARDENNE, M.; LEFRANCIER, P. & BACH, J.f., 1985. Etude de la résonance magnétique nucléaire de l'interaction du zinc avec la Thymuline. *C.R. Acad. Sci.* Paris, *t. 301* :471-475.

LEFRANCIER, P.; DERIEN, J.L.; AMIOT, J.L. & CHOAY, J., 1984. Large sclase synthesis of serum thymic factor (FTS) or thymulin. p. 251-258. In: *Peptides* (J. Ragnarsson Ed) Almquist and Wigzell. Int. Stockholm.

MARTINEZ, J.; BLANOT, D.; AUGER, G.; SASAKI, A. & BRICAS, E., 1980. Synthesis of analogs of the serum thymic nonapeptide 'facteur thymique sérique' (FTS). Part II. *Int. J. Peptide and Protein Research, 16* :267-279.

NAGAI, Y.; OSANAI, T. & SAKAKIBARA, K., 1982. Intensive suppression of experimental allergic encephalomyelitis (EAE) by serum thymic factor and therapeutic implications for multiple sclerosis. *J. Exp. Med., 52* :213-219.

PLEAU, J.M.; DARDENNE, M.; BLOUQUIT, Y. & BACH, J.F., 1977. Structural study of circulating thymic factor : a peptide isolated from pig serum. II. Aminoacid sequence. *J. Biol. Chem., 252* :8045-8047.

PLEAU, J.M.; DARDENNE, M.; BLANOT, D.; BRICAS, E. & BACH, J.F., 1979. Antagonistic analogue of serum thymic factor (FTS) interacting with the FTS cellular receptor. *Immunol. Letter, 1* :179-182.

PLEAU, J.M.; FUENTES, V.; MORGAT, J.M. & BACH, J.F., 1980. Specific receptor for the serum thymic factor (FTS) in lymphoblastoid cultured cell lines. *Proc. Natl. Acad. Sci. USA, 77* :2861-2865.

PLEAU, J.M.; PASQUES, D.; BACH, J.F.; CROS, C. & DRAY, F., 1978. Dosage immunologique du facteur thymique sérique (FTS). In *Radioimmunoassay and related procedures in Medicine.* International Atomic Energy Agency. *Vienne, 2* :505-510.

RINALDI-GARACI, C., JEZZI, T., BALDASSARE, A.M., DARDENNE, M., BACH, J.F., & GARACI, E., 1985. Effect of thymulin on intra-cellular cyclic nucleotides and prostaglandin E2 in peanut agglutinin-fractionated thymocytes. *Eur. J. Immunol., 15* :548-552.

SAVINO, W.; DARDENNE, M., & BACH, J.F., 1983. Thymic hormone containing the serum thymic factor (FTS or thymulin) in normal and autoimmune mice, as revealed by anti-FTS monoclonal antibodies. Relationship with Ia-bearing cells. *Clin. Exp. Immuno., 52* :1-7.

SAVINO, W.; DARDENNE, M., & BACH, J.F., 1983. Thymic hormone containing cells. III. Evidence for a feed-back regulation of the secretion of the serum thymic factor (FTS) by thymic epithelial cells. *Clin. Exp. Immunol., 52* :7-12.

SAVINO, W.; DARDENNE, M.; PAPIERNIK, M. & BACH, J.F., 1982. Thymic hormone-containing cells. Characterization and localization of serum thymic factor in young mouse thymus studied by monoclonal antibodies. *J. Exp. Med., 156* :628-634.

SAVINO, W.; GASTINEL, L.N.; WOLLFF, A.; DARDENNE M.; BACH J.F., 1985. Caractérisation immunochimique des molécules reconnues dans l'épithélium thymique humain par des anticorps monoclonaux anti-thymuline. *C.R. Acad. Sci.* Paris, *t. 301.* :577-579.

SAVINO, W.; WOLF, B.; ARATAN-SPIRE, S. & DARDENNE M., 1984. Thymic hormone containing cells. V. Fluctuations in the thyroid hormone levels in vivo can modulate the secretion of thymulin by the epithelial cells of young mouse thymus. *Clin. Exp. Immunol., 55* :629-635.

STRACHAN, R.G.; PALEVEDA, W.J.; BERGSTRAND, S.J.; NUTT, R.F.; HOLLY, F.W. & VEBER, D.F., 1979. Synthesis of a proposed thymic factor. *J. Med. Chem., 22* :586-588.

TOMAZIC, Y.J. & ELIAS, L.L., 1985. Modulation of the disease level and mitogen response in autoimmune mice by serum thymic factor. *Int. J. Immunopharmacol., 7* :313.

YOSHIDA, T.; TAKAOKI, M.; KATO, K.; TAKEUCHI, M.; NISHIMURA, O.; FUJINO, M. & KAKINUMA, A., 1984. The activity of synthetic analog of serum thymic factor (FTS) to convert mouse pre-T cells into Thy-1 positive cells. *Int. J. Immunopharmacol., 6* :141-146.