Chapter 10

Alanyl-Aminopeptidases in Human T Cells

Structures and functions

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Abstract: Inhibition of the enzymatic activity of alanyl-aminopeptidase leads to strong immunosuppression both *in vitro* and *in vivo*. Mechanisms involved include growth arrest, induction of immunosuppressive cytokines (TGF-β1), reduced expression of inflammatory or T cell stimulating cytokines (IL-2, IL-12), and modulation of T cell signalling pathways. Thus, T cells appear to represent a major cellular target for the pharmacological treatment of T cell mediated diseases by virtue of aminopeptidase inhibitor administration. Membrane (APN) and cytosol alanyl-aminopeptidase (ApPS), both implicated in a variety of cellular functions, show similar substrate specifity and inhibitor sensitivity. Furthermore, both enzymes are expressed in practically all T cell subsets, including the population of natural regulatory T cells that was shown recently to control the immunological tolerance to self-antigens. While the involvement of APN and ApPS in the pathological immune response is evident, the precise molecular mechanisms remain to be identified. The development of inhibitors specific for APN and ApPS is an attractive field of study and would allow determination of the individual contribution of either enzyme in the immune response.

Key words: aminopeptidase, inhibitors, structural model, T cell function, regulatory T cells

1. INTRODUCTION

Aminopeptidases are ubiquitously expressed exopeptidases found in both *Animalia* and *Plantae* that are implicated in essential cellular functions such

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as growth, differentiation, and development. Aminopeptidases in the main are metallopeptidases with preferably 1 or 2 zinc-ions bound to their catalytic site. According to their specific functions, individual aminopeptidases are located at the cell surface, within subcellular organelles and the cytosol, or are constituents of body fluids such as serum, cerebrospinal, pericardial, or synovial fluid. Both intracellular aminopeptidases and those bound to the cell surface (ectopeptidases) have been detected in immune cells and increasing evidence points to crucial regulatory functions of aminopeptidases during all steps of the normal or pathologic immune response. In support of this view, the administration of aminopeptidase inhibitors has been shown to compromise recognition and processing of peptide antigens, various steps of T cell activation, signal transduction, cytokine production, and proliferation, as well as growth, differentiation, and function of other immune cell subsets both *in vitro* and *in vivo*. The inhibition of ubiquitously expressed aminopeptidases such as methionyl-aminopeptidase supposedly impairs vital cellular functions not restricted to immune cells. In contrast, inhibitors of membrane alanyl-aminopeptidase seem to suppress the immune response via interfering specifically with CD13-positive mononuclear or T cells. Different aminopeptidases exhibit overlapping substrate specificity and inhibitor sensitivity *in vitro*. Thus, enzymatic activities determined solely by analysing the cleavage of amino acid derivatives of p-nitroanilide or β-naphthylamide substrates could be hardly assigned to individual aminopeptidase species. The availability of specific antibodies and molecular approaches considerably improved our knowledge on the tissue specificity of aminopeptidase expression. However, physiological ligands and specific functions of the leukocyte-derived enzymes *in vivo* still remained to be elucidated fully. Aminopeptidases expressed in/on immune cells are summarised in Table 1. As far as T lymphocytes are concerned, both the membrane (APN, CD13) and cytosolic (cAAP, PSA, ApPS) alanyl-aminopeptidases appear to be promising targets for the pharmacological treatment of T cell-mediated diseases. Therefore, this review is focused on these two aminopeptidases in particular.

2. MEMBRANE ALANYL-AMINOPEPTIDASE

2.1 Characteristics and Functions

Alanyl aminopeptidase (aminopeptidase N, APN, mAAP, CD13, EC 3.4.11.2) is a 966 amino acid (N-terminal Met excluded) type II

transmembrane protein expressed on the cell surface of a broad variety of cells. The zinc-dependent metalloprotease represents the prototype of the M1 family of peptidases, clan MA (gluzincins) (Hooper 1994; Rawlings and Barrett 1993). It preferentially cleaves neutral amino acids off the N-terminus of oligopeptides such as neuropeptides or growth factors. The human APN gene was cloned in 1989 and subsequently mapped to chromosome 15(q25-q26) (Look *et al.* 1989; Watt and Willard 1990). The work of Lerche and co-workers showed that the 3560 bp of coding sequence are distributed over 20 exons (Lerche *et al.* 1996).

APN is most strongly expressed in the intestine and in the kidney, and, to a lower extent, also in a number of other tissues (Barnes *et al.* 1997; Lucius *et al.* 1995). Within the hematopoietic system, APN is predominantly expressed on cells of the myelo-monocytic lineage. Therefore, anti-CD13 monoclonal antibodies are used as routine markers in the classification of human myeloid leukemias. Mature B and resting T cells lack APN expression detectable by standard flow cytometry. However, APN mRNA could be consistently detected in resting T cells and APN/CD13 expression is markedly up-regulated in response to T cell activation (Lendeckel *et al.* 1996, 1997a, 1997b).

In addition, both acute (B-ALL) and chronic (B-CLL) B cell leukaemia show abnormal APN surface expression, which in the case of adult B-ALL has been associated with a poor prognosis (Dreno *et al.* 1990; Drexler *et al.* 1991; Guyotat *et al.* 1990; Matsushita *et al.* 1994; Pinto *et al.* 1991).

The expression of the CD13 antigen on the surface of T cells stimulated by concanavalin A was first reported by Ansorge et al. (Ansorge *et al.* 1991) and Kunz and co-workers (Kunz *et al.* 1993). Later it was shown by others that a significant fraction of T cells derived from local sites of inflammation is also CD13-positive (Riemann *et al.* 1993, 1994a).

APN gene transcription is initiated at either a myeloid or an epithelial promoter, which are separated from each other by an 8 kb intron (Olsen *et al.* 1991; Shapiro *et al.* 1991). APN transcripts from myeloid cells are 259 bp longer than those from epithelial cells, but they differ in the non-coding region only. In the myeloid promoter, where transcription is initiated at a set of GC-boxes, members of the Ets and Myb families of transcription factors control APN expression (Hedge *et al.* 1998; Shapiro 1995; Shapiro *et al.* 1991; Yang *et al.* 1998). The epithelial promoter, containing a regular TATA-box, is located adjacent to the translation initiation site and includes consensus binding sites, for e.g. LF-A1, LF-B1, LF-B2 and LF-C (Olsen *et al.* 1991, 1995; Shapiro *et al.* 1991). An enhancer region of about 300 bp that is localized 2.7 kb upstream of the epithelial promoter seems to enhance the activity of both promoters (Olsen *et al.* 1997).

Table 1. Aminopeptidases of human immune cells

Amino-peptidase	Other names	Cell type / tissue	Cellular localization	Induction of expression by	References
Leu-AP	EC 3.4.11.1 LAP	T, PNC, M (U937)	cytosol, cytoplasm	PHA, ConA, IFNγ	Lundgren *et al.* 1975 Rautenberg *et al.* 1984 Kohno, Kanno 1985 Beninga *et al.* 1998
Arg-AP	EC 3.4.11.6 APB	T, M, Jurkat,	Cytosol	ConA, PHA	Rautenberg *et al.*
X-Pro-AP	EC 3.4.11.9 APP	PNC, M, T	Cytosol	PHA	Hendriks *et al.* 1991 Rusu *et al.* 1992
X-Pro-AP	EC 3.4.11.9 APP	T	cell surface		Lasch *et al.* 1998
cAAP	EC 3.4.11.14 PSA, ApPS	M, U937	Cytosol		Erbeznik, Hersh 1997
		CD4, CD8, Treg	Cytosol		Bukowska *et al.* 2003
		T	Cytosol	PHA	Komoda *et al.* 2001
mAAP	EC 3.4.11.2 APN, CD13	M, U937, THP1	cell surface	IFNγ IL-4	Ashmun *et al.* 1990 Van Hal *et al.* 1992
		T, Th1, Th2, CD4, CD8, Treg	cell surface	aCD3, aCD28, PHA, PWM, ConA, PMA	Lendeckel *et al.* 1994, 1996, 1997, 2002 Bukowska *et al.* 2003
		CD4, CD8	cell surface		Miller *et al.* 1994a,b
		DC, B	cell surface		Woodhead *et al.* 2000 Hansen *et al.* 1993 Dong *et al.* 2000
Glu-AP	EC 3.4.11.7 APA BP-1/6C3	B (mouse)	cell surface		Wu *et al.* 1991 Welch 1995
MetAP1	EC 3.4.11.18	ubiquitous	Cytoplasm		Bradshaw & Arfin 1996
MetAP2	EC 3.4.11.18	germinal center B cells	Cytoplasm		Kanno *et al.* 2002
Bleomycin-hydrolase	PepC, Gal6	ubiquitous, leukemia	Cytosol		Brömme *et al.* 1996 Ferrando *et al.* 1996
PILS-AP	adipocyte-derived LAP	spleen, thymus	ER	IFNγ	Schomburg *et al.* 2000 Serwold *et al.* 2002
IRAP	EC 3.4.11.3	B (spleen)	Cytoplasm		Wright *et al.* 1995
LTA4 hydrolase	EC 3.4.11.-	G, Mϕ	Cytoplasm, Nucleus	IL-4, IL-13	Brock *et al.* 2001 Zaitsu *et al.* 2000

T=T cells, M=monocyte, Mϕ=macrophage, G=granulocyte, B=B cell, Treg=regulatory T cell, PNC=polymorphonuclear cell, DC=dendritic cell, Th1 and Th2= T helper cell populations, CD4 T helper cells, CD8= cytotoxic T cells, IRAP=insulin-regulated aminopeptidase, PILS-AP= puromycin-insensitive leucyl-specific AP, LTA_4 hydrolase=leukotriene A_4 hydrolase

APN is supposed to be involved in the degradation of neuropeptides (Ahmad *et al.* 1992; Furuhashi *et al.* 1988; Giros *et al.* 1986; Miller *et al.* 1994a, 1994b; Mizutani *et al.* 1993; Shibanoki *et al.* 1991; Shimamura *et al.* 1988, 1991; Ward *et al.* 1990), cytokines and immunomodulatory peptides (Hoffmann *et al.* 1993; Kanayama *et al.* 1995; Mathe 1987), and angiotensins (Chansel *et al.* 1998; Palmieri *et al.* 1989; Palmieri *et al.* 1985). Furthermore, APN may contribute in extracellular matrix degradation (Fujii *et al.* 1995; Saiki *et al.* 1993) and antigen processing (Hansen *et al.* 1993; Larsen *et al.* 1996). APN also functions as a receptor for corona viruses (Delmas *et al.* 1992, 1993, 1994; Yeager *et al.* 1992) and CMV (Giugni *et al.* 1996; Söderberg *et al.* 1993).

Recent work (Bhagwat *et al.* 2001) identified APN as a selective marker of neovascularization and, thus, APN inhibitor application emerged as a powerful anti-angiogenic strategy for the treatment of e.g. cancer. In studies aimed at the identification of peptides that home specifically to solid tumors it was observed that the NGR motif binds to the endothelium of angiogenic vasculature (Pasqualini *et al.* 1995). Further investigation identified APN, which is absent from normal vasculature, as the receptor for this peptide motif (Pasqualini *et al.* 2000). APN expression on primary endothelial cells and corresponding cell lines is up-regulated in response to hypoxia and angiogenic growth factors (bFGF, VEGF). Of note, angiogenic tube formation was inhibited by exposing endothelial cells to APN inhibitors (Bhagwat *et al.* 2001). This subject is dealt with in detail in Chapter 9.

2.2 Expression of APN in Human T Cells

Resting T cells have been regarded as "APN-negative", since they lack CD13 surface expression detectable by standard flow cytometry. However, APN mRNA could be consistently detected in freshly isolated peripheral T cells by RT-PCR. An activation-dependent increase of APN gene and surface expression as well as Ala-pNA-hydrolysing activity in response to T cell activation *in vitro* has been reported (Ansorge *et al.* 1991; Kunz *et al.* 1993; Lendeckel *et al.* 1996, 1997a, 1997b, 1999; Wex *et al.* 1995), which apparently is due to both a stabilization of mRNA and increased promoter activity (Wex *et al.* 1995). Probably as a result of an activation *in vivo*, T cells derived from synovial fluid of patients suffering from rheumatoid arthritis (Riemann *et al.* 1993), or from pericardial fluid of patients with various heart diseases (Riemann *et al.* 1994a) also showed significant APN/CD13 expression. A similar induction of APN expression has been described for tumor-infiltrating T cells (Riemann *et al.* 1994b).

There are divergent data on the time-course of APN induction. In a co-cultivation model of human tonsillar T cells with synovial cells or

endothelial cells, Riemann et al. (Riemann *et al.* 1997) observed an induction of APN expression on T cells already 30 minutes after cell-cell contact. This rapid induction of APN-mRNA may rely on the fact that, similar to thymocytes, tonsillar T cells abundantly express c-Myb mRNA (Yokota *et al.* 1987). In human T cells, we observed maximum APN mRNA expression 3 and 4 days after activation (Lendeckel *et al.* 1996), which is in complete agreement with the time-course observed for the mRNA induction of c-Myb. This transcription factor plays a crucial role in the activation of the myeloid APN promoter (Hedge *et al.* 1998). Freshly isolated T cells hardly express c-Myb, but mRNA levels dramatically increase within 48 hours after T cell activation (Hirai and Sherr 1996; Shipp and Reinherz 1987; Lendeckel *et al.* 2001).

Activation-dependent induction of CD13 surface expression results from both rapid (independent of *de-novo* biosynthesis) and slow (requires biosynthesis) mechanisms (Lendeckel *et al.* 1997a). Similarly, stimulation of human leukocytes with anaphylatoxin C5a increased CD13 surface expression of granulocytes and monocytes within minutes. The induction was also resistant to inhibitors of protein biosynthesis (Werfel *et al.* 1991).

APN expression has been proven at the mRNA level in various T cell subsets including unfractionated T cells, $CD4^+$, $CD8^+$, Th1, Th2, and $CD4^+CD25^+$ regulatory T (Treg) cells (Bukowska *et al.* 2003; Lendeckel *et al.* 2002, 2003).

T cell fractions enriched for either Th1 or Th2 cells were generated by repeated stimulation of peripheral T cells by *Staphylococcus* enterotoxin A (SEA) and IL-2 in the presence of IL-4 or neutralizing anti-IL-4 mab, respectively, over a 10 day culture period. The Th1 fraction, characterized by a strong expression of IL-2 and IL-18 receptor (mRNA and protein secreted into the culture medium), showed significantly elevated APN mRNA levels and higher APN enzymatic activity, in comparison to the Th2 population (Lendeckel *et al.* 2002).

Basic APN mRNA contents were highest in Treg and $CD8^+$ cells. In response to the T cell activation, there were profound changes in the cellular amounts of APN transcripts; the extent and direction of these changes varied considerably depending on the individual cell type or activation mode. The overall induction of APN-mRNA expression in unfractionated T cells (Lendeckel *et al.* 1996) appears to be due to the increase in $CD8^+$ cells in which the expression level exceeds those of the other T cell subsets investigated (Bukowska *et al.* 2003). Therefore, the actual number of $CD8^+$ T cells in a given T cell fraction might account for considerable variations between different donors or samples. Surprisingly, there was an activation-dependent decline in APN mRNA levels in $CD4^+$ and $CD4^+CD25^+$ Treg cells. This decline may account for the decrease observed in the overall $CD4^+$ T cell fraction.

Aminopeptidase inhibitors applied simultaneously with the T cell stimulants partially prevented the induction of APN mRNA expression in peripheral T cells and also partially reversed the down-regulation of APN mRNA levels in $CD4^+$ and $CD4^+CD25^+$ Treg cells. Application of PAQ-22, a selective inhibitor of ApPS (Komoda *et al.* 2001), led to a further induction of APN in Treg cells. However, neither phebestin nor PAQ-22 was capable of changing the APN mRNA content of activated $CD8^+$ cells, although there was a tendency towards a further enhancement of APN expression. It can be concluded, therefore, that the main cellular targets of inhibitors of alanyl-aminopeptidase are $CD4^+$ cells, including Treg cells, which have gained much attention in recent years.

It has become evident that different types of regulatory T cells are capable of exerting peripheral immunological tolerance (Sakaguchi and Sakaguchi 2000; Shevach *et al.* 2001). Natural regulatory T cells ($CD4^+CD25^+$) comprise about 5-10% of unstimulated $CD4^+$ cells and they function as active suppressors of $CD4^+CD25^-$ T cells. This suppression is mediated initially by cell-cell contact and is due to a high-level expression of surface-bound TGF-β1 (Nakamura *et al.* 2001). Of note, aminopeptidase inhibitors have been shown to further increase expression levels of TGF-β1 in Treg cells (Lendeckel *et al.* 2003). Malfunction of Treg cells leads to a breakdown in immunological tolerance and, thus, to the development of autoimmune diseases such as Crohn's disease or type I diabetes (Read *et al.* 2000; Salomon *et al.* 2000; Singh *et al.* 2001).

2.3 APN Inhibitors Affect Leukocyte Growth and Function

Most studies that made use of APN inhibitors demonstrated their strong anti-proliferative activity towards various cell types, including leukocytes. APN inhibitors of different specificity and, thus, efficacy have been applied in both *in vitro* and *in vivo* studies. The most widely used unselective aminopeptidase inhibitor bestatin (Ubenimex) affected the growth of various cell types *in vitro* (Ino *et al.* 1991, 1992; Sakurada *et al.* 1990). In addition, bestatin inhibited the growth of transplanted tumour cells in a mouse model (Inoi *et al.* 1995; Kowalski *et al.* 1995) as well as of gastrointestinal tumour or T cell leukaemia cells in man (Iwahashi *et al.* 1994a; Okamura *et al.* 1992; Yamagishi *et al.* 1991). These antiproliferative effects were mediated by both direct cytotoxic effects (Inoi *et al.* 1995; Okamura *et al.* 1992) and the activation of macrophages, NK cells or $CD8^+$ T cells (Iwahashi *et al.* 1994a, 1994b; Yamagishi *et al.* 1991). Early reports showed a stimulation by bestatin of the proliferation and DNA-synthesis of human T cells, Concanavalin A-stimulated T-cells, or mouse splenocytes

(Ishizuka *et al.* 1980; Müller *et al.* 1979; Saito *et al.* 1976), but these data are in contrast to those of others (Lendeckel *et al.* 1996, 1997b, 1999; Morikawa *et al.* 1989). Bestatin, at sub-micromolar concentrations only partially inhibits APN, but shows a significant inhibition of Leu-AP and ApPS (Tieku and Hooper 1992). Actinonin (IC_{50} 2 μM), probestin (IC_{50} 50 nM), phebestin (IC_{50} 20 nM) and RB3014 (IC_{50} 15 nM) appear to be more effective inhibitors of APN (Aoyagi *et al.* 1990; Chen *et al.* 1999; Nagai *et al.* 1997; Tieku and Hooper 1992; Yoshida *et al.* 1990), and were also capable of inhibiting the growth of human T cells, mononuclear cells, and of the T cell lines KARPAS-299 and H9 (Lendeckel *et al.* 1996, 1997b, 1998).

In our hands, the unspecific aminopeptidase inhibitor bestatin as well as the supposedly more specific APN inhibitors, actinonin and probestin, caused a dose-dependent decrease of DNA synthesis of activated peripheral T cells or mononuclear cells, of the T cell lines KARPAS-299, P12/Ichikawa, H9, and of the pro-myeloid cell line U937 (Lendeckel *et al.* 1996, 1998).

The inhibition of APN expression by antisense-oligonucleotides resulted in a similar reduction of DNA synthesis of activated peripheral T cells, T cell lines H9 and KARPAS-299 as well as of U937 cells. This strongly implies that indeed the inhibition of APN caused the growth inhibition observed in response to inhibitor administration.

A fact that needs to be considered is that these growth inhibitory effects could be observed in both $CD13^+$ (U937, Karpas-299, activated T cells) and $CD13^-$ negative cells (H9). Thus, at present it could not be fully excluded that the inhibition of other aminopeptidases contributes to the observed decrease of cellular proliferation. One such candidate aminopeptidase is the ubiquitously expressed cytosol alanyl-aminopeptidase (ApPS). This cytosolic aminopeptidase is hardly distinguishable from APN with respect to substrate and inhibitor specificity. In addition, inhibition of ApPS enzymatic activity by puromycin resulted in a growth inhibition similar to that observed in response to decreasing APN expression or activity (Constam *et al.* 1995).

It should be kept in mind, however, that "CD13-negative" cells harbour considerable amounts of APN mRNA, which at least in the case of the T cell lines are equal to those of typical $CD13^+$ cells. Therefore, the existence of an intracellular pool of APN has been proposed (Lendeckel *et al.* 1997a).

A number of studies addressed the question whether the anti-proliferative effects of APN inhibitors were mediated by changes of cytokine or cytokine receptor expression. Bestatin was reported to increase the concentration of GM-CSF in the culture medium of mononuclear cells (Okamura *et al.* 1990) and to stimulate M-CSF receptor expression of U937 cells (Murata *et al.* 1994). We could show that the antisense-mediated inhibition of APN expression in U937 cells increased the amounts of IL-1-

receptor antagonist (IL-1RA) detectable in the culture medium, but decreased that of TNF-α (Wex *et al.* 1997). Activation of human mononuclear cells led to a rapid increase of IL-1β mRNA amounts within 24 hours, that could be effectively reduced by the aminopeptidase inhibitors actinonin, probestin, and leuhistin. This decrease of IL-1β expression could also be observed at the protein level (unpublished). Probestin was shown to reduce the activation-dependent increase of IL-2 mRNA in human peripheral T cells (Lendeckel *et al.* 1999).

A possible key step for mediating the anti-proliferative effects resulting from APN inhibition is the increase of the expression and secretion of the highly potent immunosuppressive cytokine, TGF-β1. Both actinonin and probestin were shown to be capable of inducing TGF-β1 expression in human mononuclear cells (MNC), peripheral T cells, and Treg cells (Lendeckel *et al.* 1999, 2002). In a number of cell types TGF-β1 provokes a cell cycle arrest in G_1 via an inhibition of expression and/or activity of the cyclin-dependent kinases (CDK) CDK4 and CDK2 (Ewen 1996). Among the genes controlled by TGF-β1 are those of inhibitors of CDKs p21/Waf-1 (Dkhissi *et al.* 1999; Hunt *et al.* 1998), p27/kip (Dkhissi *et al.* 1999; Mahmud *et al.* 1999), and p15/INK4B (Ewen 1996; Li *et al.* 1997). RB3014 provoked an induction of the transcription factor Sp1 in human peripheral T cells (Lendeckel *et al.* 2002). Since the genes of both p21/Waf-1 and TGF-β1 themself are responsive to Sp1 (Geiser *et al.* 1993; Han *et al.* 2001; Koutsodontis *et al.* 2001; Udvadia *et al.* 1993), these results provide a reasonable mechanism how APN inhibition might interfere with the cell cycle machinery.

Table 2. Inhibition of APN and ApPS by various aminopeptidase inhibitors

	IC_{50}-Values (μM)	
Inhibitor	**ApPS** [a]	**APN** [b]
phebestin	0.015	0.5
probestin	0.016	0.05 [c]
RB 3014	0.025	0.02
actinonin	0.075	0.79
PAQ-22	0.29	no inhibition
bestatin	0.4	89.1 [c]
puromycin	3.1	90 [d]
iodacetamide	97.5	no inhibition

[a] from human kidney, [b] from H9 cells, [c] Tieku and Hooper 1992, [d] Brownlees and Williams 1993.

2.4 Natural Peptide Hormones as Inhibitors of APN

The work of Xu and co-workers (Xu *et al.* 1995) identified the neuropeptides substance P and bradykinin as natural inhibitors of APN. Since both peptides exhibit a proline residue in their penultimate N-terminal position it was hypothesised that this particular proline is an essential structural requirement for peptides inhibitory to APN. To verify these assumptions we investigated the inhibitory capacity towards APN of various X-Pro-peptides. Among the X-Pro-peptides analysed, substance P appeared to be the most potent inhibitor of human APN. The K_i value determined (0.62 μM) was in accordance to that described by Xu et al. (Xu *et al.* 1995). APN activity was inhibited with decreasing efficiency by other peptides in the order substance P(1-4) > ranatachykinin > bradykinin. β-casomorphin and α-MSH lacked any inhibitory activity. This strongly implies that the presence of a P2-proline is not a sufficient feature to give a potent aminopeptidase inhibitor. Interestingly, replacement of the N-terminal part of bradykinin by the N-terminal RPKP-motif of substance P greatly enhanced the inhibitory capacity of bradykinin. Of note, all peptides that actually exhibited APN inhibitory activity contain an additional proline residue either in P4 or P3 position. Our data suggest the (K/R)P(K/R)P-motif conferring inhibitory activity to a peptide, probably in context with a basic amino acid, preferably in P3-position. Accordingly, ranatachykinin that consists of a RPSP-N-terminal motif appeared to be half as potent an inhibitor as substance P (Bukowska *et al.*, unpublished).

2.5 Structural Model of APN

In the absence of any crystal structure of APN molecular modelling analyses were performed. The APN model obtained included amino acids from Tyr^{335} to Gln^{607} and identified APN as a curved structure (Bukowska *et al.*, unpublished). Both histidine residues of the $H^{387}ELAH$ motif, glutamate Glu^{388}, and the carbonyl oxygen of the peptide co-ordinately bind the Zn^{2+} of APN. In addition, the model showed that amino acids Phe^{348}, Asp^{432}, and Trp^{428} are crucial determinants of ligand affinity, particularly of substance P-like structures. Phe^{348} established interactions to proline residues in the inhibitor molecule and Asp^{432} forms salt bridges preferentially to N-terminal basic amino acids. Trp^{428}, which marks the beginning of the binding pocket, shows hydrophobic interactions with the side chain of lysine K3 and proline P4. Binding studies of substance P were made by manual dockings and with the program GOLD (Jones *et al.* 1997), with both methods predicting pK_Ds in the same range. In any test, substance P appeared to be the best inhibitor molecule.

Binding of bradykinin to the catalytic site of APN seems to be mediated by interactions of the N-terminal arginine R1 with Asp^{432}, of proline P2 with Phe^{348}, and of proline P3 and glycine G4 with the Trp^{428} (Figure 1). These two proline residues are capable of building a so-called sandwich structure. This option, however, has not been investigated in more detail. Furthermore, phenylalanine F5 is orientated towards the side chain of receptor (APN) residue Arg^{380}.

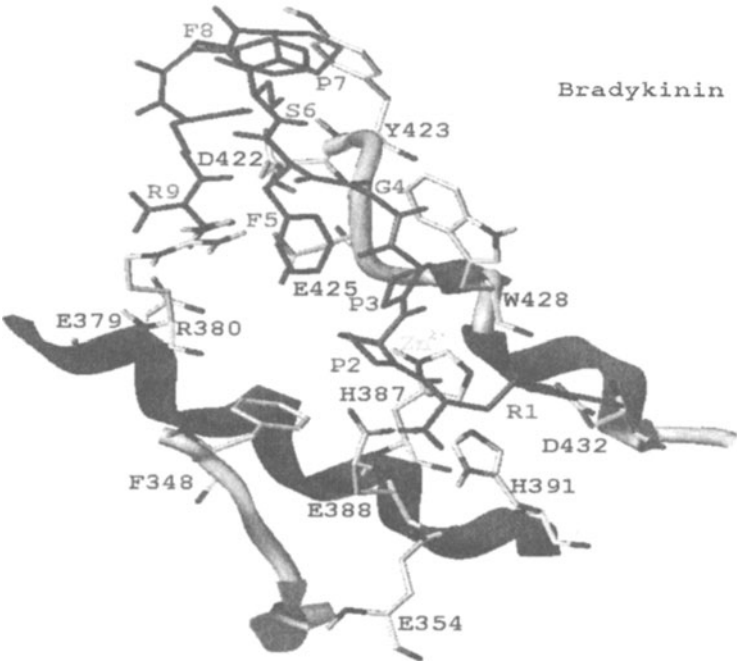

Figure 1. Docking arrangement of bradykinin into the active site of APN

This, as well as the favoured arrangements of proline P7 towards Tyr^{423} and of arginine R9 towards Glu^{379}, however, causes with respect to binding to APN an unfavourable orientation of the amino acid residues serine S6 and phenylalanine F8.

The worse pK_D of bradykinin when compared to that of substance P could be explained by the fact that the hydrophobic phenylalanine F8 of bradykinin is directed towards the solvent.

Table 3. Comparison between (by SCORE) predicted pK_D scores and pK_I scores from literature (Xu, Wellner, and Scheinberg, 1995)

peptide	K_I [μM]	pK_I	pK_D (from SCORE)	difference from pK_D to pK_I
substance P	0.44	6.36	6.33	- 0.5 %
bradykinin	9.4	5.03	4.90	- 2.6 %
morphiceptin	169	3.77	5.95	+ 57.8 %

The established model was applied to predict highly potent inhibitory peptides by using LIGBUILDER (Wang *et al.* 2000). Peptide ligands designed accordingly exhibited good pK_D values throughout and perfectly fit into the binding pocket via the interactions described above. A substructure common to all designed ligands was a proline residue arranged towards Phe^{348}. Of all the ligands designed the highest pK_d-value of 9.4 was predicted for the one shown in Figure 2. However, none of the proposed ligands has been verified experimentally, as yet.

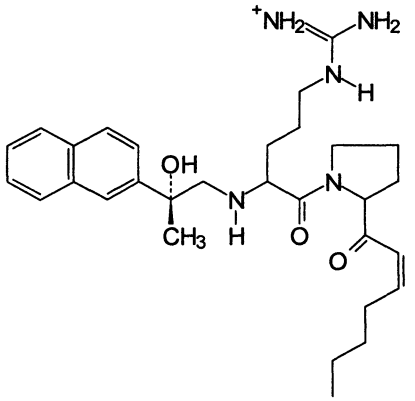

Figure 2. Proposed structure for a new APN-specific inhibitor.

APN is type II transmembrane protein attached to the cells surface via its N-terminally located transmembrane anchor. As shown by electron microscopy (Hussain *et al.* 1981), it is predominantly expressed as a homodimer. About 100 different arrangements of the homodimers were generated by protein-protein dockings using GRAMM (Vakser 1997). These arrangements could be divided into three families (Figure 3). About one third of the dimers could be assigned to family 1. In this case the arrangement of the monomers to each other is so, that their N- or C-termini are located at the same site. In contrast, structures belonging to the other

families show antiparallel arrangements of the N- and C-termini. Therefore, only those belonging to the first family may represent dimer arrangements likely to occur *in vivo*.

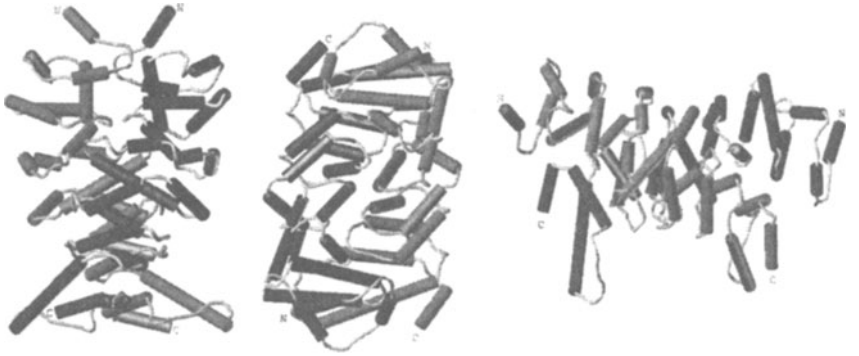

Figure 3. Representative structures of the monomer arrangements predicted by GRAMM (from left to right: family 1, 2, 3). Only the structure of family 1 is supposed to represent a physiological one.

Interestingly, family 1 dimer models imply the existence of a roll-over mechanism: in different dimers the monomers appear to be differently positioned to each other. This implies that one monomer rolls over the other leading to a dislocation of the binding pockets which thereby could be either opened or closed in a dynamic process (Figure 4).

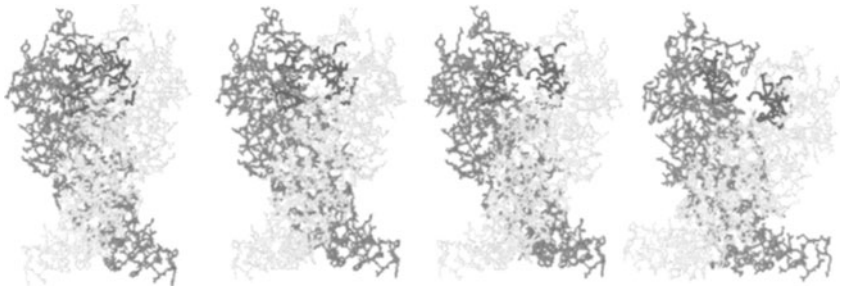

Figure 4. Schematic representation of a dynamic opening and closing of the active site (black) by movement of the two monomers (light and dark grey) to each other.

2.6 Mechanisms of APN Signaling

A few ectopeptidases present on leukocyte surfaces are supposed to be capable of inducing or modulating signal transduction (for review see Goding and Howard 1998; Riemann *et al.* 1999). Emerging evidence attributes a "signaling" function to APN/CD13 as well. Early work showed that bestatin changes activity and cellular localization of PKC in K562 cells (Kumano and Sugawara 1992). In U937 cells, bestatin increased the activity of the Pp60/c-Src tyrosine-kinase (Murata *et al.* 1994). A very recent study aimed at the identification of potential targets mediating the anti-proliferative effects of alanyl-aminopeptidase expression and activity revealed a modulation of MAP kinase p42/Erk2 activity and mRNA-levels in KARPAS-299 cells by probestin and actinonin (Lendeckel *et al.* 1998a). Different time courses observed for p42/Erk2-activation and its mRNA induction suggest that both mechanisms function independently of each other. A phosphorylation (activation) of Erk1/2 could also be observed after ligation of APN/CD13 by anti-CD13 mab in human monocytes (Santos *et al.* 2000).

The involvement of the wnt-pathway in mediating cellular effects resulting from APN inhibition was implied by the observed up-regulation of Wnt-5a expression by actinonin in peripheral human T cells (Lendeckel *et al.* 2000a). Furthermore, expression and activity of glycogen-synthase kinase-3β (GSK-3β), an inherent component of the Wnt-signaling pathway, were found to be increased in the course of T cell activation (Lendeckel *et al.* 2000b). These changes were not restricted to special T cell mitogens, but rather depend on an effective T cell activation. Several APN inhibitors, including the highly effective compound RB3014 partially reversed these activation-dependent changes of GSK-3β expression and activity.

Wnt-signals increase the phosphorylation of GSK-3β at Ser9 and, thereby inactivate this negative regulatory protein kinase. This prevents substrates such as β-catenin, eIF2B or cyclin D1 from being phosphorylated and, thus, controls their activity or rescues them from proteasome-mediated degradation (Aberle *et al.* 1997; Diehl *et al.* 1998). Consequently, there is a stimulation of e.g. TCF-mediated transcription, protein biosynthesis, and cell cycle progression. In addition to the regulation by Wnt-5a, GSK-3β activity is regulated by PKB, which itself is controlled or modulated by e.g. MAP kinases, PKA, PI3-kinase and, interestingly, signalling from the T cell co-stimulatory molecule CD28.

Inhibition of GSK-3β by Li^+ caused a sustained cell cycle arrest at G2/M transition in endothelial cells without compromising cell viability (Mao *et al.* 2001). Interestingly, Li^+ increased the expression of the CDK inhibitor, p21, depending on the presence of p53, which is indicative of an activation by GSK-3β of the p53 pathway. Remarkably, an inhibitor of

methionyl aminopeptidase I, TNP-470, provokes cell cycle arrest of endothelial cells in late G_1, only in the presence of p21 and p53 (Yeh *et al.* 2000).

The upstream mechanisms that initiate the observed changes in T cell signalling, proliferation, and cytokine production in response to the administration of aminopeptidase inhibitors remain to be identified.

3. EXPRESSION OF ApPS IN T CELL SUBSETS

The cytosol alanyl-aminopeptidase (cAAP, PSA, ApPS, EC 3.4.11.14) is described in detail in Chapter 1. Here, special focus is on the expression and possible functions of ApPS in human immune cells, particularly in T cells and subsets thereof.

ApPS and also leucine-aminopeptidase reportedly participate in the post-proteasomal cleavage of the N-terminus of antigenic peptides (Beninga *et al.* 1998; Stoltze *et al.* 2000; Saric *et al.* 2001; Levy *et al.* 2002; Saveanu *et al.* 2002). Interferon-γ can stimulate the post-proteasomal cleavage of the N-terminus of antigenic peptides by inducing the production of leucine aminopeptidase *in vitro* (Beninga *et al.* 1998). ApPS is not regulated by interferon-γ and, in contrast to our results, its expression has been described to be constitutive (Saric *et al.* 2001).

The full understanding of the mechanisms underlying the immunosuppressive activity of aminopeptidase inhibitors has been hampered by the fact that a number of aminopeptidases are equally sensitive to non-discriminating aminopeptidase inhibitors and share near identical substrate specificities. This is especially true for APN and ApPS (Table 2), which, until the recent development of the ApPS-specific, thalidomide-derived substances PIQ-22, PAQ-22, and PAZOX-22 (Komoda *et al.* 2001; Kakuta *et al.* 2001), could not be inhibited selectively. Phebestin (Nagai *et al.* 1997) and RB3014 (Chen *et al.* 1999) are generally considered as highly potent and selective inhibitors of APN. These substances also inhibit ApPS enzymatic activity with equal - or even higher - efficiency. Furthermore, we demonstrate that anti-CD13 monoclonal antibodies directed towards the highly conserved catalytic site of APN, show considerable cross-reactivity to ApPS (Figure 5). This is in line with previously published data (Murray *et al.* 1993) showing the existence of intracellular anti-CD13 immunoreactivity in $CD13^-$ Jurkat cells after fixation of the cells. Thus, previous studies that made use of non-selective aminopeptidase inhibitors or cross-reactive antibodies are difficult to interpret with respect to the specific enzyme that has been targeted and, thus, is responsible for the effects observed.

Expression of ApPS could be demonstrated at the mRNA level in human peripheral T cells and in all T cell subsets investigated, including $CD4^+$, $CD8^+$, Th1, Th2 and $CD4^+CD25^+$ Treg cells. Basic ApPS mRNA contents did not differ among the T cell subsets. However, in response to T cell activation there were profound changes in cellular ApPS mRNA amounts detectable (Bukowska *et al.* 2003). In all T cell subsets investigated, except for Treg cells, activation provoked a strong increase in the amount of ApPS mRNA. In addition to APN and ApPS, other T cell aminopeptidase species also appear to be highly inducible (see Table 1).

Inhibitors of the enzymatic activity of APN and/or ApPS strongly modulated expression of ApPS in unfractionated peripheral T cells and in most T cell subsets. Using phebestin or PAQ-22, the amount of ApPS mRNA was increased in $CD4^+$ and $CD4^+CD25^+$ cells, but decreased in unfractionated T cells. Expression of ApPS in $CD8^+$ cells appeared to be hardly affected by aminopeptidase inhibition. Since the ApPS-selective inhibitor PAQ-22 affected ApPS expression less potently than the non-discriminating inhibitor phebestin, inhibition of APN itself rather than that of ApPS seems to be the cause of changes in ApPS mRNA expression in T cell populations. Similarily, APN mRNA levels were more strongly affected by non-discriminating inhibitors of alanyl-aminopeptidases, with the exception of Treg cells. In the latter cells, the selective inhibition of ApPS provoked a strong increase in APN expression.

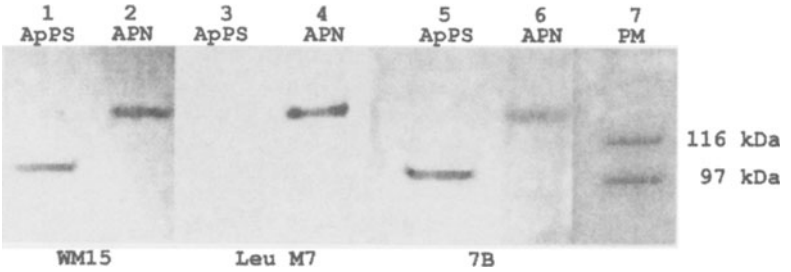

Figure 5. Immunoblot analysis of ApPS and APN. 500 ng each of ApPS from the human T cell line H9 (lanes 1, 3, and 5) and APN from human kidney (lanes 2, 4, and 6) were subjected to SDS-PAGE under non-reducing conditions and blotted onto nitrocellulose membrane. Immunodetection was performed by using the anti-CD13 monoclonal antibodies clone WM15 (lanes 1, 2) and clone LeuM7 (lanes 3, 4) as well as the polyclonal antiserum 7B (protein A-purified Ig from rabbit raised against the synthetic peptide KERVVTVIA HELAHQ representing the catalytic site of ApPS. Note the considerable cross-reactivity of WM15 and 7B with ApPS or APN, respectively. (PM = molecular weight marker)

The existence of functionally polarised $CD4^+$ cell responses is based on their profile of cytokine secretion and the preferential expression of distinct

activation markers and transcriptions factors (Romagnani 1999). Human Th1 cells develop in response to intracellular bacteria, some viruses and cytolytic agents. In contrast, human Th2 cells develop in response to allergens and helminth components, which are responsible for strong antibody production and eosinophil activation, but lack cytolytic activity (Romagnani 1992, 1999). The Th1 and Th2 cells play different roles in protection against exogenous agents and also in immunopathology. The cytokine milieu in the microenvironment of Th-precursors determines their differentiation into Th1 or Th2 subsets (De Carli *et al.* 1994). In this study we exploited the fact that presence of IL-4 favours the development of Th2 cells *in vitro*, whereas its absence determines the Th1 phenotype (Romagnani 1992; Parronchi *et al.* 1992; Maggi *et al.* 1992). The comparison of ApPS mRNA expression between Th1 and Th2 cells revealed a higher expression level in Th2 cells, a finding that might be indicative of a specific role for ApPS in the Th2-mediated response. Notably, Th1 cells showed higher APN expression and activity when compared to Th2 cells. Thus, selective inhibitors of either ApPS or APN might prove valuable tools for the treatment of Th2- or Th1-dominated diseases, respectively.

4. CONCLUSIONS

The data discussed here support the hypothesis that balanced expression and enzymatic activities of APN and ApPS in/on cells of the immune system play a role in the regulation and/or modulation of leukocyte growth and function. Accumulating evidence also suggests the capability of special (patho)-physiological conditions such as malignant transformation, inflammation, T-cell activation, autoimmune disease, and allograft rejection of modulating the expression of APN and ApPS in human T lymphocytes themselves. Thereby, T cells emerged as targets for an alternative therapy of both T cell-mediated or T cell-dominated diseases of both the Th1 and Th2 type.

Although our understanding of the molecular and cellular mechanisms that mediate the anti-proliferative and immunosuppressive effects resulting from inhibition of APN or ApPS is still in the very beginning, the observed modulation of GSK-3β activity and TGF-β1 expression could represent essential key steps.

Potent and selective inhibitors of ApPS have become available during the last years. Hopefully, selective inhibition of APN will be another option in the near future. This would allow to study the individual roles both enzymes play in regulating the normal and pathologic immune response and would

provide a rationale for their usage as powerful pharmacological tools in the treatment of T cell-mediated diseases.

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REFERENCES

Aberle, H., Bauer, A., Stappert, J., Kispert, A., Kemler, R., 1997, beta-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J.* **16:** 3797-3804.

Ahmad, S., Wang, L., Ward, P.E., 1992, Dipeptidyl(amino)peptidase IV and aminopeptidase M metabolize circulating substance P in vivo. *J. Pharmacol. Exp. Ther.* **260:** 1257-1261.

Ansorge, S., Schön, E., Kunz, D., 1991, Membrane-bound peptidases of lymphocytes: functional implications. *Biomed. Biochim. Acta* **50:** 799-807.

Aoyagi T., Yoshida S., Nakamura Y., Shigihara Y., Hamada M., Takeuchi T., 1990, Probestin, a new inhibitor of aminopeptidase M, produced by streptomyces azureus MH663-2F6. *J. Antibiotics* **XLIII:** 143-148.

Ashmun, R.A. and Look, A.T., 1990, Metalloprotease activity of CD13/aminopeptidase N on the surface of human myeloid cells. *Blood* **75:** 462-469.

Barnes, K., Walkden, B.J., Wilkinson, T.C., Turner, A.J., 1997, Expression of endothelin-converting enzyme in both neuroblastoma and glial cell lines and its localization in rat hippocampus. *J. Neurochem.* **68:** 570-577.

Belhacene, N., Mari, B., Rossi, B., Auberger, P., 1993, Characterization and purification of T lymphocyte aminopeptidase B: a putative marker of T cell activation. *Eur. J. Immunol.* **23:** 1948-1955.

Beninga, J., Rock, K.L., Goldberg, A.L., 1998, Interferon-gamma can stimulate post-proteasomal trimming of the N terminus of an antigenic peptide by inducing leucine aminopeptidase. *J. Biol. Chem.* **273:** 18734-18742.

Bhagwat, S. V., Lahdenranta, J., Giordano, R., Arap, W., Pasqualini, R., Shapiro, L.H., 2001, CD13/APN is activated by angiogenic signals and is essential for capillary tube formation. *Blood* **97:** 652-659.

Bradshaw, R.A. and Arfin, S.M., 1996, Methionine aminopeptidase: structure and function, In: The Aminopeptidases, vol. 6 (Taylor, A., ed.) Georgetown: R.G. Landes Co., pp. 91-106.

Brock, T.G., Maydanski, E., McNish, R.W., Peters-Golden, M., 2001, Co-Localization of leukotriene A4 hydrolyse with 5-lipoxygenase in nuclei of alveolar macrophages and rat basophilic leukemia cells but not neutrophils. *J. Biol. Chem.* **276:** 35071-35077.

Brömme, D., Rossi, A.B., Smeekens, S.P., Anderson, D.C., Payan, D.G., 1996, Human bleomycin hydrolase: molecular cloning, sequencing, functional expression, and enzymatic characterization. *Biochem.* **35:** 6706-6714.

Brownlees, J., Williams, C.H., 1993, Peptidases, peptides and the mammalian blood-brain barrier. *J. Neurochem.* **60:** 793-803.

Bukowska, A., Tadje, J. Arndt, M., Wolke, C., Kähne, T., Bartsch, J., Faust, J., Neubert, K., Hashimoto, Y., Lendeckel, U., 2003, Transcriptional regulation of cytosol and membrane alanyl-aminopeptidase in human T cell subsets. *Biol. Chem.* **384:** 657-665.

Chansel, D., Czekalski, S., Vandermeersch, S., Ruffet, E., Fournie-Zaluski, M.C., Ardaillou, R., 1998, Characterization of angiotensin IV-degrading enzymes and receptors on rat mesangial cells. *Am. J. Physiol.* **275:** F535-F542.

Chen, H., Roques, B. P., Fournie-Zaluski, M.-C., 1999, Design of the first highly potent and selective aminopeptidase N (EC3.4.11.2) inhibitor. *Bioorg. Med. Chem. Letters* **9:** 1511-1516.

Constam, D.B., Tobler, A.R., Rensing-Ehl, A., Kemler, I., Hersh, L.B., Fontana, A., 1995, Puromycin-sensitive aminopeptidase. Sequence analysis, expression, and functional characterization. *J. Biol. Chem.* **270:** 26931-26939.

De Carli, M., D'Elios, M.M., Zancuoghi, G., Romagnani, S., Del Prete, G., 1994, Human Th1 and Th2 cells: functional properties, regulation of development and role in autoimmunity. *Autoimmunity* **18:** 301-308.

Delmas, B., Gelfi, J., Kut, E., Sjöström, H., Noren, O., Laude, H., 1994, Determinants essential for the transmissible gastroenteritis virus-receptor interaction reside within a domain of aminopeptidase-N that is distinct from the enzymatic site. *J. Virol.* **68:** 5216-5224.

Delmas, B., Gelfi, J., L'Haridon, R., Vogel, L.K., Sjöström, H., Noren, O., Laude, H., 1992, Aminopeptidase N is a major receptor for the entero-pathogenic coronavirus TGEV. *Nature* **357:** 417-420.

Delmas, B., Gelfi, J., Sjöström, H., Noren, O., Laude, H., 1993, Further characterization of aminopeptidase-N as a receptor for coronaviruses. *Adv. Exp. Med. Biol.* **342:** 293-298.

Diehl, J. A., Cheng, M., Roussel, M.F., Sherr, C.J., 1998, Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev.* **12:** 3499-3511.

Dkhissi, F., Raynal, S., Lawrence, D. A., 1999, Altered complex formation between p21waf, p27kip and their partner G1 cyclins determines the stimulatory or inhibitory transforming growth factor-1 growth response of human fibroblasts. *Int. J. Oncol.* **14:** 905-910.

Dong, X., An, B., Salvucci Kierstead, L., Storkus, W.J., Amoscato, A.A., Salter, R.D., 2000, Modification of the amino terminus of a class II epitope confers resistance to degradation by CD13 on dendritic cells and enhances presentation to T cells. *J. Immunol.* **164:** 129-135.

Dreno, B., Bureau, B., Stalder, J.F., Litoux, P., 1990, MY7 monoclonal antibody for diagnosis of cutaneous T-cell lymphoma. *Arch. Dermatol.* **126:** 1454-1456.

Drexler, H. G., Thiel, E., Ludwig, W.-D., 1991, Review of the incidence and clinical relevance of myeloid antigen-positive acute lymphoblastic leukemia. *Leukemia* **5:** 637-645.

Erbeznik, H. and Hersh, L.B., 1997, A novel mammalian high-molecular-weight aminopeptidase. *Arch. Biochem. Biophys.* **1:** 228-234.

Ewen, M. E., 1996, p53-dependent repression of cdk4 synthesis in transforming growth factor-beta-induced G1 cell cycle arrest. Journal of Laboratory and Clinical Medicine **128:** 355-360.

Ferrando, A.A., Velasco, G., Campo, E., Lopez-Otin, C., 1996, Cloning and expression analysis of human bleomycin hydrolase, a cysteine proteinase involved in chemotherapy resistance. *Cancer Res.* **56:** 1746-1750.

Fujii, H., Nakajima, M., Saiki, I., Yoneda, J., Azuma, I., Tsuruo, T., 1995, Human melanoma invasion and metastasis enhancement by high expression of aminopeptidase N/CD13. *Clin. Exp. Metastasis* **13:** 337-344.

Furuhashi, M., Mizutani, S., Kurauchi, O., Kasugai, M., Narita, O., Tomoda, Y., 1988, In vitro degradation of opioid peptides by human placental aminopeptidase M. *Exp. Clin. Endocrinol.* **92:** 235-237.

Geiser, A. G., Busam, K. J., Kim, S. J., Lafiyatis, R., O'Reilly, M. A., Webbink, R., Roberts, A. B., Sporn, M. B., 1993, Regulation of the transforming growth factor-beta1 and -beta3 promoters by transcription factor Sp1. *Gene* **129:** 223-228.

Giros, B., Gros, C., Solhonne, B., Schwartz, J.C., 1986, Characterization of aminopeptidases responsible for inactivating endogenous (Met5)enkephalin in brain slices using peptidase inhibitors and anti-aminopeptidase M antibodies. *Mol. Pharmacol.* **29:** 281-287.

Giugni, T. D., Söderberg, C., Ham, D.J., Bautista, R.M., Hedlund, K.-O., Möller, E., Zaia, J.A., 1996, Neutralization of human cytomegalovirus by human CD13-specific antibodies. *J. Infect. Diseases* **173:** 1062-1071.

Goding, J. W. Howard, M.C., 1998, Ecto-enzymes of lymphoid cells. *Immunol. Rev.* **161:** 5-10.

Grdisa, M. and Vitale, L., 1991, Types and localization of aminopeptidases in different human blood cells. *Int. J. Biochem.* **23:** 339-345.

Guyotat, D., Campos, L., Shi, Z.H., Charrin, C., Treille, D., Magaud, J.P., Fiere, D., 1990, Myeloid surface antigen expression in adult acute lymphoblastic leukemia. *Leukemia* **4:** 664-666.

Han, J. W., Ahn, S.H., Kim, Y.K., Bae, G.U., Yoon, J.W., Hong, S., Lee, H.Y., Lee, Y.W., Lee, H.W., 2001, Activation of p21superWAF1/Cip1 transcription through Sp1 sites by histone deacetylase inhibitor apicidin: Involvement of protein kinase C. *J. Biol. Chem.* **276:** 42084-42090.

Hansen, A.S., Noren, O., Sjöström, H., Werdelin O., 1993, A mouse aminopeptidase N is a marker for antigen-presenting cells and appears to be co-expressed with major histocompatibility complex class II molecules. *Eur. J. Immunol.* **23:** 2358-2364.

Hedge, S. P., Kumar, A., Kurschner, C., Shapiro, L.H., 1998, c-Maf interacts with c-Myb to regulate tanscription of an early myeloid gene during differentiation. *Mol. Cell. Biol.* **18:** 2729-2737.

Hendriks, D., De Meester, I., Umiel, T., Vanhoof, G., van Sande, M., Scharpe, S., Yaron, A., 1991, Aminopeptidase P and dipeptidyl peptidase IV activity in human leukocytes and in stimulated lymphocytes. *Clin. Chim. Acta* **196:** 87-96.

Hirai, H. and Sherr, C.J., 1996, Interaction of D-type cyclins with a novel myb-like transcription factor, DMP1. *Mol. Cell. Biol.* **16:** 6457-6467.

Hoffmann, T., Faust, J., Neubert, K., Ansorge, S., 1993, Dipeptidyl peptidase IV (CD 26) and aminopeptidase N (CD 13) catalyzed hydrolysis of cytokines and peptides with N-terminal cytokine sequences. *FEBS Lett.* **336:** 61-64.

Hooper, N.M., 1994, Families of zinc metalloproteases. *FEBS Lett.* **354:** 1-6.

Hunt, K. K., Fleming, J. B., Abramian, A., Zhang, L., Evans, D. B., Chiao, P. J., 1998, Overexpression of the tumor suppressor gene Smad4/DPC4 induces p21waf1 expression and growth inhibition in human carcinoma cells. *Cancer Res.* **58:** 5656-5661.

Hussain, M.M., Tranum-Jensen, J., Norén, O., Sjöström, H., Christiansen, K., 1981, Reconstitution of purified amphiphilic pig intestinal microvillus aminopeptidase. Mode of membrane insertion and morphology. *Biochem. J.* **199:** 179-186.

Ino K., Goto S., Kosaki A., Nomura S., Asada E., Misawa T., Furuhashi Y., Mizutani S., a., Tomoda Y., 1991, Groth inhibitory effect of bestatin on choriocarcinoma cell lines in vitro. *Biotherapy* **3:** 351-357.

Ino, K., Isobe, K., Goto, S., Nakashima, I., Tomoda, Y., 1992, Inhibitory effect of bestatin on the growth of human lymphocytes. *Immunopharmacol.* **23:** 163-171.

Inoi, K., Goto, S., Nomura, S., Isobe, K., Nawa, A., Okamoto, T., Tomoda, Y., 1995, Aminopeptidase inhibitor ubenimex (bestatin) inhibits the growth of human choriocarcinoma in nude mice through its direct cytostatic activity. *Anticancer Res.* **15:** 2081-2087.

Ishizuka, M., Sato, J., Sugiyama, Y., Takeuchi, T., Umezawa, H., 1980, Mitogenic effect of bestatin on lymphocytes. *J. Antibiotics (Tokyo)* **33:** 653-662.

Iwahashi, M., Tanimura, H., Yamaue, H., Tani, M., Noguchi, K., Mizobata, S., Tsunoda, T., Tamai, M., Arii, K., 1994a, Ubenimex treatment enhances the susceptibility of gastric cancer cell lines to lymphokine-activated killer cells. *Anticancer Res.* **14:** 1563-1568.

Iwahashi, M., Tanimura, H., Yamaue, H., Tsunoda, T., Tani, M., Noguchi, K., Mizobata, S., Tamai, M., Hotta, T., Arii, K., 1994b, In vitro augmentation of cytotoxic activity of peripheral blood lymphocytes and spleen cells of cancer patients by ubenimex. *Anticancer Res.* **14:** 1557-1562.

Jones, G., Willett, P., Glen, R.C., Leach, A.R., Taylor, R., 1997, Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **267**: 727-748.

Kakuta, H., Koiso, Y., Takahashi, H., Nagasawa, K., Hashimoto, Y., 2001, Novel specific puromycin-sensitive aminopeptidase inhibitors: 3-(2,6-diethylphenyl)-2,4(1H,3H)-quinazolinedione and N-(2,6-diethylphenyl)-2-amino-4H-3,1-benzoxazin-4-one. *Heterocycles* **55:** 1433-1438.

Kanayama, N., Kajiwara, Y., Goto, J., el Maradny, E., Maehara, K., Andou, K., Terao, T., 1995, Inactivation of interleukin-8 by aminopeptidase N (CD13). *J. Leukoc. Biol.* **57:** 129-134.

Kanno, T., Endo, H., Takeuchi, K., Morishita, Y., Fukayama, M., Mori, S., 2002, High expression of methionine aminopeptidase type 2 in germinal center B cells and their neoplastic counterparts. Lab. Invest. 82: 893-901.

Kohno, H. and Kanno, T., 1985, Intracellular localization and properties of aminopeptidases in human peripheral lymphocytes. *Int. J. biochem.* **17:** 1143-1148.

Kohno, H. and Kanno, T., 1985, properties and activities of aminopeptidases in normal and mitogen-stimulated human lymphocytes. *Biochem. J.* **226:** 59-65.

Komoda, M., Kakuta, H., Takahashi, H., Fujimoto, Y., Kadoya, S., Kato, F., Hashimoto, Y., 2001, Specific inhibitor of puromycin-sensitive aminopeptidase with a homophthalimide skeleton: identification of the target molecule and a structure-activity relationship study. *Bioorg. Med. Chem.* **9:** 121-131.

Koutsodontis, G., Tentes, I., Papakosta, P., Moustakas, A., Kardassis, D., 2001, Sp1 plays a critical role in the transcriptional activation of the human cyclin-dependent kinase inhibitor p21(WAF1/Cip1) gene by the p53 tumor suppressor protein. *J. Biol. Chem.* **276:** 29116-29125.

Kowalski, J., Belowski, D., Madej, A., Herman, Z.S., 1995, Antitumor activity of bestatin and thiorphan in mice. *Arch Immunol Ther Exp (Warsz)* **43:** 265-271.

Kumano, N. and Sugawara, S., 1992, Ubenimex (Bestatin), an aminopeptidase inhibitor, modulates protein kinase C in K562 cells. *J. Biol. Regul. Homeost. Agents* **6:** 116-120.

Kunz, D., Bühling, F., Hütter, H.J., Aoyagi, T., Ansorge, S., 1993, Aminopeptidase N (CD13, EC 3.3.4.11.2) occurs on the surface of resting and concanavalin A-stimulated lymphocytes. *Biol. Chem. Hoppe-Seyler* **374:** 291-296.

Larsen, S. L., Pedersen, L. O., Buus, S., Stryhn, A., 1996, T cell responses affected by aminopeptidase N (CD13)-mediated trimming of major histocompatibility complex class II-bound peptides. *J. Exp. Med.* **184:** 183-189.

Lasch, J., Moschner, S., Sann, H., Zellmer, S., Koelsch, R., 1998, Aminopeptidase P is a cell surface antigen of endothelial and lymphoid cells: catalytic and immunohistotopical evidences. *Biol. Chem.* **379:** 705-709.

Lendeckel, U., Wex, Th., Reinhold, D., Kähne, Th., Frank, K., Faust, J., Neubert, K., Ansorge, S., 1996, Induction of the membrane alanyl aminopeptidase gene and surface expression in human T-cells by mitogenic activation. *Biochem. J.* **319:** 817-823.

Lendeckel, U., Wex, T., Reinhold, D., Arndt, M., Ittenson, A., Frank, K., Ansorge, S., 1997, Activation-dependent induction of T cell alanyl aminopeptidase and its possible involvement in T cell growth, in: *Adv. Exp. Med. Biol.* **421:** 59-66.

Lendeckel, U., Wex, T., Ittenson, A., Arndt, M., Frank, K., Mayboroda, I., Schubert, W., Ansorge, S., 1997a, Rapid mitogen-induced aminopeptidase N surface expression in human T cells is dominated by mechanisms independent of de novo protein biosynthesis. *Immunobiol.* **197:** 55-69.

Lendeckel, U., Kähne, T., Arndt, M., Frank, K., Ansorge, S., 1998, Inhibition of alanyl aminopeptidase induces MAP-kinase p42/ERK2 in the human T cell line KARPAS-299. *Biochem. Biophys. Res. Commun.* **252:** 5-9.

Lendeckel, U., Wex, T., Arndt, M., Frank, K., Franke, A., Ansorge, S., 1998, Identification of point mutations in the aminopeptidase N gene by SSCP analysis and sequencing. *Hum. Mutation* **Suppl. 1:** S158-S160.

Lendeckel, U., Arndt, M., Frank, K., Wex, T., Ansorge, S., 1999, Role of alanyl aminopeptidase in growth and function of human T cells (review). *Int. J. Mol. Med* **4:** 17-27.

Lendeckel, U., Arndt, M., Frank, K., Spiess, A., Reinhold, D., Ansorge, S., 2000a, Modulation of WNT-5A expression by actinonin: linkage of APN to the WNT-pathway? *Adv. Exp. Med. Biol.* **477:** 35-41.

Lendeckel, U., Scholz, B., Arndt, M., Frank, K., Spiess, A., Chen, H., Roques, B.P., Ansorge, S., 2000b, Inhibition of alanyl-aminopeptidase suppresses the activation-dependent induction of glycogen synthase kinase-3beta (GSK-3beta) in human T cells. *Biochem. Biophys. Res. Commun.* **273:** 62-65.

Lendeckel, U., Arndt, M., Firla, B., Wolke, C., Wex, T., Ansorge, S., 2002, CD13/APN in hematopoietic cells – expression, function, regulation, and clinical aspects. pp 25-49. In: Ectopeptidases. CD13/Aminopeptidase N and CD26/Dipeptidylpeptidase IV in Medicine and biology. (Langner J and Ansorge S; Eds.) Kluwer Academic/Plenum Publishers; New York, Boston, Dordrecht, London Moscow.

Lendeckel, U., Arndt, M., Bukowska, A., Tadje, J., Wolke, C., Kähne, T., Neubert, K., Faust, J., Ittenson, A., Ansorge, S., Reinhold, D., 2003, Synergistic action of DPIV and APN in the regulation of T cell function. pp123-131, In: Advances in Experimental Medicine and Biology, Vol. 524 Dipeptidyl aminopeptidases in Health and disease. (Hildebrandt M, Klapp B, Hoffmann T, Demuth H-U, eds.) Kluwer Academic / Plenum Publishers, New York, Boston, Dordrecht, London, Moscow.

Lerche, C., Vogel, L.K., Shapiro, L.H., Noren, O., Sjöström, H., 1996, Human aminopeptidase N is encoded by 20 exons. *Mammalian Genome* **7:** 712-713.

Levy, F., Burri, L., Morel, S., Peitrequin, A.L., Levy, N., Bachi, A., Hellman, U., Van den Eynde, B.J., Servis, C., 2002, The final N-terminal trimming of a subaminoterminal proline-containing HLA class I-restricted antigenic peptide in the cytosol is mediated by two peptidases. *J. Immunol.* **169:** 4161-4171.

Li, J. M., Hu, P. P., Shen, X., Yu, Y., Wang, X. F. 1997, E2F4-RB and E2F4-p107 complexes suppress gene expression by transforming growth factor beta through E2F binding sites. *Proc. Natl. Acad. Sci. U.S.A.* **94:** 4948-4953.

Look, A. T., Ashmun, R.A., Shapiro, L.H., Peiper, S.C., 1989, Human myeloid plasma membrane glycoprotein CD13 (gp150) is identical to aminopeptidase N. *J. Clin. Invest.* **83:** 1299-1307.

Lucius, R., Sievers, J., Mentlein, R., 1995, Enkephalin metabolism by microglial aminopeptidase N (CD13). *J. Neurochem.* **64:** 1841-1847.

Lundgren, E., Roos, G., Tärnik, A., 1975, Subcellular distribution of amino acis naphthylamidase isozymes in human leukocytes. *Acta Pathol. Microbiol. Scand.* **A 83:** 176-182.

MacArthur, M.W. and Thornton, J.M., 1991, Influence of proline residues on protein conformation. *J. Mol. Biol.* **218:** 397-412.

Maggi, E., Parronchi, P., Manetti, R., Simonelli, C., Piccinni, M.P., Rugiu, F.S., De Carli, M., Ricci, M., Romagnani, S., 1992, Reciprocal regulatory effects of IFN-gamma and IL-4 on the in vitro development of human Th1 and Th2 clones. *J. Immunol.* **148:** 2142-2147.

Mahmud, N., Katayama, N., Nishii, K., Sugawara, T., Komada, Y., Mitani, H., Araki, H., Ohishi, K., Watanabe, M., Masuya, M., Nishikawa, M., Minami, N., Ohashi, H., Shiku, H., 1999, Possible involvement of bcl-2 in regulation of cell-cycle progression of haemopoietic cells by transforming growth factor-beta1. Br. J. Haematol. 105: 470-477.

Mao, C. D., Hoang, P., DiCorleto, P.E., 2001, Lithium inhibits cell cycle progression and induces stabilization of p53 in bovine aortic endothelial cells. *J. Biol. Chem.* **276:** 26180-26188.

Mathe, G., 1987, Do tuftsin and bestatin constitute a biopharmacological immunoregulatory system? *Cancer Detect. Prevent. Suppl.* **1:** 445-455.

Matsushita, A., Nagai, K., Ishikawa, T., Tatsumi, E., Ohno, Y., Takahashi, T., 1994, B-cell prolymphocytic leukemia expressing CD13 antigen. *Int. J. Hematol.* **60:** 157-161.

Miller, B. C., Ackroyd, A., Hersh, L.B., Cottam, G.L., 1994a, Methionine enkephalin metabolism by murine macrophage ectopeptidase(s). *Regul. Pept.* **50:** 87-98.

Miller, B. C., Thiele, D.L., Hersh, L.B., Cottam, G.L., 1994b, Methionine enkephalin is hydrolyzed by aminopeptidase N on CD4+ and CD8+ spleen T cells. *Arch. Biochem. Biophys.* **311:** 174-179.

Mizutani, S., Goto, K., Nomura, S., Ino, K., Goto, S., Kikkawa, F., Kurauchi, O., Goldstein, G., Tomoda, Y., 1993, Possible action of human placental aminopeptidase N in feto-placental unit. *Res. Commun. Chem. Pathol. Pharmacol.* **82:** 65-80.

Morikawa K., Morikawa S., Nakano A., Oseko F., 1989, Bestatin, an inhibitor of aminopeptidase B, suppresses the proliferation and differentiation of human B-cells in vitro. *Int. J. Immunopharmacol.* **11:** 905-913.

Müller, W. E., Zahn, R.K., Arendes, J., Munsch, N., Umezawa, H., 1979, Activation of DNA metabolism in T-cells by bestatin. *Biochem. Pharmacol.* **28:** 3131-3137.

Murata, M., Kubota, Y., Tanaka, T., Iida Tanaka, K., Takahara, J., Irino, S., 1994, Effect of ubenimex on the proliferation and differentiation of U937 human histiocytic lymphoma cells. *Leukemia* **8:** 2188-2193.

Murray, H., Turner, A.J., Kenny, A.J., 1994, The aminopeptidase activity in the human T-cell lymphoma line (Jurkat) is not at the cell surface and is not aminopeptidase N (CD-13). *Biochem. J.* **298:** 353-360.

Nagai, M., Kojima, F., Naganawa, H., Hamada, M., Aoyagi, T., Takeuchi, T., 1997, Phebestin, a new inhibitor of aminopeptidase N, produced by Streptomyces sp. MJ716-m3. *J. Antibiotics* **50:** 82-84.

Nakamura, K., Kitani, A., Strober, W., 2001, Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J. Exp. Med.* **194:** 629-644.

Okamura, S., Omori, F., Haga, K., Baba, H., Sugimachi, K., Niho, Y., 1990, Influence of bestatin on production of granulocyte-macrophage colony-stimulating factor from human peripheral blood mononuclear cells in vitro. *Acta Oncologica* **29:** 795-797.

Okamura, T., Shibuya, T., Harada, M., Niho, Y., 1992, Successful treatment of chronic adult T-cell leukemia with ubenimex. *Acta Haematologica* **87:** 94-97.

Olsen, J., Kokholm, K., Troelsen, T., Laustsen, L., 1997, An enhancer with cell-type dependent activity is located between the myeloid and epithelial aminopeptidase N (CD13) promoters. *Biochem. J.* **322:** 899-908.

Olsen, J., Laustsen, L., Karnstrom, U., Sjostrom, H., Noren, O., 1991, Tissue-specific interactions between nuclear proteins and the aminopeptidase N promoter. *J. Biol. Chem.* **266:** 18089-18096.

Palmieri, F. E., Bausback, H.H., Ward, P.E., 1989, Metabolism of vasoactive peptides by vascular endothelium and smooth muscle aminopeptidase M. *Biochem. Pharmacol.* **38:** 173-180.

Palmieri, F. E., Petrelli, J.J., Ward, P.E., 1985, Vascular, plasma membrane aminopeptidase M. Metabolism of vasoactive peptides. *Biochem. Pharmacol.* **34:** 2309-2317.

Parronchi, P., De Carli, M., Manetti, R., Simonelli, C., Sampognaro, S., Piccinni, M.P., Macchia, D., Maggi, E., Del Prete, G., Romagnani, S., 1992, IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. *J. Immunol.* **149:** 2977-2983.

Pasqualini, R., Koivunen, E., Ruoslahti, E., 1995, A peptide isolated from phage display libraries is a structural and functional mimic of an RGD-binding site on integrins. *J. Cell Biol.* **130:** 1189-1196.

Pasqualini, R., Koivunen, E., Kain, R., Lahdenranta, J., Sakamoto, M., Stryhn, A., Ashmun, R.A., Shapiro, L.H., Arap, W., Ruoslahti, E., 2000, Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis. *Cancer Res.* **60:** 722-727.

Pinto, A., Del Vecchio, L., Carbone, A., Roncadin, M., Volpe, R., Serraino, D., Monfardini, S., Colombatti, A., Zagonel, V., 1991, Expression of myelomonocytic antigens is associated with unfavourable clinicoprognostic factors in B-cell chronic lymphocytic leukaemia. *Ann. Oncol.* **2 Suppl 2:** 107-113.

Rautenberg, W. and Tschesche, H., 1984, Aminopeptidases from human leukocytes. *Hoppe-Seyler's Z. Physiol. Chem.* **365:** 49-58.

Rawlings, N.D. and Barrett, A.J., 1993, Evolutionary families of peptidases. *Biochem. J.* **290:** 205-218.

Read, S., Malmström, V., Powrie, V., 2000, Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J. Exp. Med.* **192:** 295-302.

Riemann, D., Schwachula, A., Hentschel, M., Langner, J., 1993, Demonstration of CD13/aminopeptidase N on synovial fluid T cells from patients with different forms of joint effusions. *Immunobiol.* **187:** 24-35.

Riemann, D., Göhring, B., Langner, J., 1994a, Expression of aminopeptidase N/CD13 in tumour-infiltrating lymphocytes from human renal cell carcinoma. *Immunol. Lett.* **42:** 19-23.

Riemann, D., Wollert, H.G., Menschikowski, J., Mittenzwei, S., Langner, J., 1994b, Immunophenotype of lymphocytes in pericardial fluid from patients with different forms of heart disease. *Int. Arch. Allergy Immunol.* **104:** 48-56.

Riemann, D., Kehlen, A., Thiele, K., Löhn, M., Langner, J., 1997, Co-incubation of lymphocytes with fibroblast-like synoviocytes and other cell types can induce lymphocytic surface expression of aminopeptidase N/CD13. *Adv. Exp Med. Biol.* **421:** 75-79.

Riemann, D., Kehlen, A., Langner, J., 1999, CD13 - not just a marker in leukemia typing. *Immunol. Today* **20:** 83-88.

Romagnani, S., 1995, Biology of human TH1 and TH2 cells. *J. Clin. Immunol.***15:** 121-129.

Romagnani, S., 1999, Th1/Th2 cells. Inflamm. Bowel Dis. 5: 285-294.

Rusu, I. and Yaron, A., 1992, Aminopeptidase P from human leukocytes. *Eur. J. Biochem.* **210:** 93-100.

Saiki, I., Fujii, H., Yoneda, J., Abe, F., Nakajima, M., Tsuruo, T., Azuma, I., 1993, Role of aminopeptidase N (CD13) in tumor-cell invasion and extracellular matrix degradation. *Int. J. Cancer* **54:** 137-143.

Saito, M., Aoyagi, T., Umezawa, H., Nagai, Y., 1976, Bestatin, a new specific inhibitor of aminopeptidases, enhances activation of small lymphocytes by concanavalin A. *Biochem. Biophys. Res. Commun.* **76:** 526-533.

Sakaguchi, S., and Sakaguchi, N., 2000, Role of genetic factors in organ-specific autoimmune diseases induced by manipulating the thymus or T cells, and not self-antigens. *Rev. Immunogenet.* **2:** 147-153.

Sakurada K., Imamura M., Kobayashi M., Tachibana N., Abe K., Tanaka M., Okabe M., Morioka M., Kasai M., Sugiura T., 1990, Inhibitory effect of bestatin on the growth of human leukemic cells. *Acta Oncologica* **29:** 799-802.

Salomon, B., Lenschow, D.J., Rhee, L., Ashourian, N., Singh, B., Sharpe, A., Bluestone, J.A., 2000, B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* **12:** 431-440.

Santos, A. N., Langner, J., Herrmann, M., Riemann, D., 2000, Aminopeptidase N/CD13 is directly linked to signal transduction pathways in monocytes. *Cell Immunol.* **201:** 22-32.

Saric, T., Beninga, J., Graef, C.I., Akopian, T.N., Rock, K.L., Goldberg, A.L., 2001, Major histocompatibility complex class I-presented antigenic peptides are degraded in cytosolic extracts primarily by thimet oligopeptidase. *J. Biol. Chem.* **276:** 36474-36481.

Saveanu, L., Fruci, D., van Endert, P., 2002, Beyond the proteasome: trimming, degradation and generation of MHC class I ligands by auxiliary proteases. *Mol. Immunol.* **39:** 203-215.

Schomburg, L., Kollmus, H., Friedrichsen, S., Bauer K., 2000, Molecular charactrization of a puromycin-insensitive leucyl-specific aminopeptidase, PILS-AP. *Eur. J. Biochem.* **267:** 3198-3207.

Serwold, T., Gonzalez, F., Kim, J., Jacob, R., Shastri, N., 2002, ERAAP customizes peptides for MHC class I molecules in the endoplasmatic reticulum. *Nature* **419:** 480-483.

Shapiro, L. H., 1995, Myb and Ets proteins cooperate to transactivate an early myeloid gene. *J. Biol. Chem.* **270:** 8763-8771.

Shapiro, L. H., Ashmun, R.A., Roberts, W.M., Look, A.T., 1991, Separate promoters control transcription of the human aminopeptidase N gene in myeloid and intestinal epithelial cells. *J. Biol. Chem.* **266:** 11999-12007.

Shevach, E.M., McHugh, R.S., Piccirillo, C.A., Thornton, A.M., 2001, Control of T-cell activation by CD4+ CD25+ suppressor T cells. *Immunol. Rev.***182:** 58-67.

Shibanoki, S., Weinberger, S.B., Ishikawa, K., Martinez, J.L., Jr., 1991, Further characterization of the in vitro hydrolysis of [Leu]- and [Met]enkephalin in rat plasma: HPLC-ECD measurement of substrate and metabolite concentrations. *Regulat. Peptides* **32:** 267-278.

Shimamura, M., Hazato, T., Iwaguchi, T., 1988, A new aminopeptidase in monkey cerebral membrane fraction: hydrolysis of enkephalin. *Brain Res.* **445:** 350-353.

Shimamura, M., Hazato, T., Iwaguchi, T., 1991, Enkephalin-degrading aminopeptidase in the longitudinal muscle layer of guinea pig small intestine: its properties and action on neuropeptides. *J. Biochem. Tokyo.* **109:** 492-497.

Shipp, M. A. and Reinherz, E.L., 1987, Differential expression of nuclear proto-oncogenes in T cells triggered with mitogenic and nonmitogenic T3 and T11 activation signals. *J. Immunol.* **139:** 2143-2148.

Singh, B., Read, S., Asseman, C., Mottet, C., Stephens, L.A., Stepankova, R., Tlaskalova, H., Powrie, F., 2001, Control of intestinal inflammation by regulatory T cells. *Immunol. Rev.* **182:** 190-200.

Söderberg, C., Giugni, T.D., Zaia, J.A., Larsson, S., Wahlberg, J.M., Möller, E., 1993, CD13 (human aminopeptidase N) mediates human cytomegalovirus infection. *J. Virol.* **67:** 6576-6585.

Stoltze, L., Schirle, M., Schwarz, G., Schroter, C., Thompson, M.W., Hersh, L.B., Kalbacher, H., Stevanovic, S., Rammensee, H.G., Schild, H., 2000, Two new proteases in the MHC class I processing pathway. *Nat. Immunol.* **1:** 413-418.

Tieku, S. and Hooper, N.M., 1992, Inhibition of aminopeptidases N, A, and W. *Biochem. Pharmacol.* **44:** 1725-1730.

Udvadia, A. J., Rogers, K. T., Higgins, P. D., Murata, Y., Martin, K. H., Humphrey, P. A., Horowitz, J. M., 1993, Sp-1 binds promoter elements regulated by the RB protein and Sp-1-mediated transcription is stimulated by RB coexpression. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 3265-3269.

Vakser, I.A., 1997, Evaluation of GRAMM low-resolution docking methodology on the hemagglutinin antibody- complex. Proteins 1: 226-230.

Van Hal, P.T., Hopstaken-Broos, J.P., Wijkhuijs, J.M., Te Velde, A.A., Figdor, C.G., Hoogsteden, H.C., 1992, Regulation of aminopeptidase –N (CD13) and Fc epsilon RIIb (CD23) expression by IL-4 depends on the stage of maturation of monocytes/macrophages. J. Immunol. 149: 1395-1401.

Wang, R., Gao, Y., Lai, L.,. 2000, LigBuilder: A Multi-Purpose Program for Structure-Based Drug Design. J. Mol. Model 6: 498-516.

Ward, P. E., Benter, I.F., Dick, L., Wilk, S., 1990, Metabolism of vasoactive peptides by plasma and purified renal aminopeptidase M. *Biochem. Pharmacol.* **40:** 1725-1732.

Watt, V. M. and Willard, H.F., 1990, The human aminopeptidase N gene: isolation, chromosome localization, and DNA polymorphism analysis. *Hum. Genet.* **85:** 651-654.

Welch, P.A., 1995, Regulation of B cell precursor proliferation by aminopeptidase A. *Int. Immunol.* **7:** 737-746.

Werfel, T., Sonntag, G., Weber, M.H., Gotze, O., 1991, Rapid increases in the membrane expression of neutral endopeptidase (CD10), aminopeptidase N (CD13), tyrosine phosphatase (CD45), and Fc gamma-RIII (CD16) upon stimulation of human peripheral leukocytes with human C5a. *J. Immunol.* **147:** 3909-3914.

Wex, T., Lendeckel, U., Reinhold, D., Kähne, T., Arndt, M., Frank, K., Ansorge, S., 1997, Antisense-mediated inhibition of aminopeptidase N (CD13) markedly decreases growth rates of hematopoietic tumour cells, in Ansorge S, Langner J. (eds): Cellular peptidases in immune function and diseases. New York, Plenum Press, pp 67-73

Wex, T., Lendeckel, U., Wex, H., Frank, K., Ansorge, S., 1995, Quantification of aminopeptidase N mRNA in T cells by competitive PCR. *FEBS Lett.* **374:** 341-344.

Woodhead, V.E., Stonehouse, T.J., Binks, M.H., Speidel, K., Fox, D.A., Gaya, A., Hardie, D., Henniker, A.J., Horejsi, V., Sagawa, K., Skubitz, K.M., Taskov, H., Todd, R.F. 3rd, van Agthoven, A., Katz, D.R., Chain, B.M., 2000, Novel molecular mechanisms of dendritic cell-induced T cell activation. *Int. Immunol.* **12:** 1051-1061.

Wright, J.W., Krebs, L.T., Stobb, J.W., Harding, J.W., 1995, The angiotensin IV system: functional implications. *Front. Neuroendocrinol.* **161:** 23-52.

Wu, Q., Li, L., Cooper, M.D., Pierres, M., Gorvel, J.P., 1991, Aminopeptidase A activity of the murine B-lymphocyte differentiation antigen BP-1/6C3. *Proc. Natl. Acad. Sci. USA* **88:** 676-680.

Xu, Y., Wellner, D., Scheinberg, D.A., 1995, Substance P and bradykinin are natural inhibitors of CD13/aminopeptidase N. *Biochem. Biophys. Res. Commun.* **208:** 664-674.

Yamagishi, H., Naito, K., Ueda, Y., Itoi, S., Kohnosu, H., Kobayashi, M., Kubo, H., Matsuda, A., Oka, T., 1991, Bestatin administration and the change in cellular immunity. *Biomed. Pharmacother.* **45:** 117-120.

Yang, C., Shapiro, L.H., Rivera, M., Kumar, A., Brindle, P.K., 1998, A role for CREB binding protein and p300 transcriptional coactivators in Ets-1 transactivation functions. *Mol. Cell. Biol.* **18:** 2218-2229.

Yeager, C. L., Ashmun, R.A., Williams, R.K., Cardellichio, C.B., Shapiro, L.H., Look, A.T., Holmes, K.V., 1992, Human aminopeptidase N is a receptor for human coronavirus 229E. *Nature* **357:** 420-422.

Yeh, J. R., Mohan, R., Crews, C.M., 2000, The antiangiogenic agent TNP-470 requires p53 and p21CIP/WAF for endothelial cell growth arrest. *Proc. Natl. Acad. Sci. U. S. A.* **97:** 12782-12787.

Yokota, S., Yuan, D., Katagiri, T., Eisenberg, R.A., Cohen, P. L., Ting, J.P., 1987, The expression and regulation of c-myb transcription in B6/lpr Lyt-2-, L3T4-T lymphocytes. *J. Immunol.* **139:** 2810-2817.

Yoshida, S., Nakamura, Y., Naganawa, H., Aoyagi, T., Takeuchi, T., 1990, Probestin, a new inhibitor of aminopeptidase M, produced by Streptomyces azureus MH663-2F6. II. Structure determination of probestin. *J. Antibiot. Tokyo.* **43:** 149-153.

Zaitsu, M., Hamasaki, Y., Matsuo, M., Kukita, A., Tsuji, K., Miyazaki, M., Hayasaki, R., Muro, E., Yamamoto, S., Kobayashi, I., Ichimura, T., Kohashi, O., Miyazaki, S., 2000, New induction of leukotriene A(4) hydrolase by interleukin-4 and interleukin-13 in human polymorpho-nuclear leukocytes. *Blood* **96:** 601-609.