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Increased Lymphocytic Aminopeptidase N/CD13 Promoter Activity After Cell-Cell Contact

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Abstract Aminopeptidase N (APN)/CD13 is a transmembrane ectoenzyme expressed on a wide variety of cells. With respect to haematopoietic cells, APN/CD13 has been considered specific for the myeloid lineage, because granulocytes and monocytes/macrophages, but not lymphocytes of peripheral blood, show a surface expression of CD13 antigen. However, we could recently show that cell-cell contact of lymphocytes with endothelial cells, monocytes, and fibroblast-like synoviocytes (SFCs) results in an increase of steady-state APN/CD13 mRNA and a rapid expression of cell-surface protein on the lymphocytes. In this study using the Dual-Luciferase reporter assay, we demonstrate that interaction of the T-cell line Jurkat with SFCs results in a higher activity of the APN/CD13 myeloid promoter in T cells. An enhancer located between the myeloid and epithelial APN/CD13 promoter increases the response of the promoter to the cell-cell contact-induced expression of APN/CD13 in lymphocytes. Adhesion of lymphocytes to extracellular matrix did not result in increased promoter activity. The lymphocytic promoter response induced by direct cell-cell contact with SFCs is not affected by mutations of a proximal promoter element (nucleotides −48 to −35), which has a possible functional role in the basal APN/CD13 gene expression in lymphocytes. Upregulated peptidase-promoter activity via cell-cell contact shown in this study for the first time is discussed as a general mechanism in peptidase induction. J. Cell. Biochem. 80:115–123, 2000.

Key words: cell-cell interactions; aminopeptidase N/CD13; T lymphocytes; fibroblast-like synoviocytes

Aminopeptidase N (APN/CD13; EC 3.4.11.2) catalyzes the removal of N-terminal, preferentially neutral, amino acid residues from small peptides [for review see Sanderink et al., 1988; Shipp and Look 1993]. This 150-kDa metalloprotease has been found in many tissues, with especially high expression in brush border membranes of kidney proximal tubules, intestine, and placenta. Sequence comparisons of the cloned cDNA showed that APN is identical to the CD13 antigen [Look et al., 1989].

For a long time, APN/CD13 has been considered a myeloid marker because this enzyme is expressed during most developmental stages of myeloid cells. Lymphocytes of peripheral blood and of tonsils show no surface expression of CD13 antigen. APN/CD13 mRNA expression, however, has been detected in leukemic T-cell lines [Lendeckel et al., 1994]. Furthermore, the CD13 antigen is frequently expressed on the surface of some lymphocytic precursor cells [Syrjala et al., 1994] as well as lymphatic leukemia cells [Dreno et al., 1990; Drexler et al., 1991; Ohsaka et al., 1996]. Our investigations have shown the presence of $CD13^+$ T lymphocytes in the synovial fluid of people with various forms of arthritis [Riemann et al., 1993], in the pericardial fluid of people undergoing thoracic surgery for heart valve replacement [Riemann et al., 1994a], and within tumor-infiltrating lymphocytes, especially of renal-cell carcinoma [Riemann et al., 1994b]. To study in more detail cellular conditions explaining the differences in APN/CD13 expression on lymphocytes of different origin, we used cocultivation assays of lymphocytes plus fibroblast-like synoviocytes (SFCs, propagated from rheumatoid pannus) as a model of cellular

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interactions in the rheumatoid joint resulting in lymphocytic APN/CD13 expression. We found that direct cell-cell contact of lymphocytes with SFCs is able to induce APN/CD13 mRNA and protein expression on T and B cells after 1 h of coincubation [Riemann et al., 1997]. There exist several other reports on regulation of surface ectopeptidase expression by cell-cell contact [reviewed in Riemann et al., 1999]. None of these examples has been studied in depth with respect to the gene regulation. The APN/CD13 gene seems well suited to such an investigation because of the rather detailed knowledge of regulatory elements. The coding part of the gene (15q25–q26) is encoded by 20 exons [Lerche et al., 1996; Watt and Willard, 1990]. Two promoters separated by approximately eight kb control the gene. A so-called "epithelial" promoter close to the coding part of the gene controls the APN transcription in intestinal epithelial cells [Olsen et al., 1991]. In fibroblasts, cells of haematopoietic origin and in T-cell lines, a so-called "myeloid" promoter is active [Shapiro et al., 1991; Lendeckel et al., 1994]. The mRNAs generated from the two different promoters differ in their 5′ untranslated regions, but encode the same protein because they share the same initiator codon methionin. The APN epithelial promoter contains a TATA box and binding sites for Sp1, members of hepatocyte nuclear factor 1 (HNF1) homeodomain family, and a member of the steroid hormone receptor family, presumably COUP-tf [Olsen et al., 1995]. The myeloid promoter is GC rich, lacks a TATA box, and contains binding sites for members of the transcription factor families Myb and Ets in the region from position −411 to −291 upstream of the major transcription-initiation site [Shapiro et al., 1991; Shapiro 1995]. Other transcription factors collaborating in myeloid APN/CD13 transcription are DMP1, c-Maf and the coactivator CBP/p300 [Hedge et al., 1998; Inoue et al., 1998; Yang et al., 1998]. An enhancer was located with activity in both epithelial and myeloid cells 2.7 kb upstream of the transcriptional start site used in epithelial cells [Olsen et al., 1997]. The enhancer contains two consensus-binding sites for members of the Ets transcription factor family, one region with a CCAAT enhancer-binding protein, and one region with Sp1. The enhancer stimulates the transcription from a heterologous promoter (SV 40 early promoter) and is active in liver, intestinal, and myeloid cells [Olsen et al., 1997].

In earlier experiments [Riemann et al., 1997], we only showed the increase in lymphocytic steady-state APN/CD13 mRNA levels upon cell-cell contact. With the aim of a better understanding of the lymphocytic APN/CD13 expression, we studied in closer detail the regulation of the basal APN/CD13 gene expression in T cells as well as the increased expression after direct cell-cell contact with SFCs. Furthermore, we asked if the enhancer located between the myeloid and epithelial APN/CD13 promoter has a role in the lymphocytic APN/CD13 gene expression after cell-cell contact or contact to extracellular matrix (ECM).

MATERIALS AND METHODS

Materials

Culture media (RPMI 1640, OptiMem), Superscript II-RT, oligonucleotides, genistein, and DMRIE-C were purchased from Gibco BRL (Karlsruhe, Germany). Fetal calf serum (FCS) was obtained from C.C.Pro (Karlsruhe, Germany). Random primer DNase I was from Boehringer (Mannheim, Germany); Taq polymerase was from Qiagen (Hilden, Germany); Dual-Luciferase reporter assay system, GeneEditor mutagenesis system, and plasmids pGL3 and pRL-TK were from Promega (Mannheim, Germany); plasmid pEGFP was from Clontech (Heidelberg, Germany); [γ-^{32}P] ATP and Hyperfim-ECL were from Amersham Live Science (Braunschweig, Germany); thermocycler and ScanPack were from Biometra (Göttingen, Germany). The luminometer Lumat LB9507 was from EG&G Berthold (Bad Wildbad, Germany). Collagen type I and IST+ premix solution were purchased from Becton Dickinson (Heidelberg, Germany), fibronectin from Biochrom (Berlin, Germany), collagenase type II from Sigma (Deisenhofen, Germany), antibodies against c-Myb and c-Ets1/2 from Santa Cruz (Heidelberg, Germany).

Cell Isolation and Culture

Human palatine tonsils were obtained from surgical specimens of patients undergoing tonsillectomy for tonsillar hypertrophy or recurrent tonsillitis. The tonsillar material was kept at 4°C in phosphate-buffered saline and processed for isolation of mononuclear cells within 3 h. Tonsils were cut into small pieces, and

then sieved under gentle pressure. Mononuclear cells were separated by the standard Ficoll-Hypaque gradient method. Plastic-adherent cells were removed from tonsillar cell suspensions by incubation of cells in RPMI 1640 containing 10% FCS for 2.5 h at 37°C. T and B cells were separated by rosetting with sheep red blood cells followed by gradient centrifugation on Ficoll-Hypaque. T cells obtained after lysis of red blood cells were >96% pure, as estimated by use of flow cytometry analysis with antibodies against CD19, CD3, and CD14. The human lymphocytic cell lines Jurkat, HUT78, and the colon carcinoma cells Caco 2 (American Type Culture Collection) were cultured in RPMI 1640 with 10% FCS, antibiotics (1 μl/ml fungizone, 0.75 μl/ml refobacin), and 50 μmol 2-mercaptoethanol. SFCs were obtained from patients with rheumatoid arthritis undergoing surgical synovectomy by dissociating the minced tissue enzymatically with Hanks' balanced salt solution containing 0.5 mg/ml collagenase type II, 0.15 mg/ml DNase I, and 5 mM Ca^{2+}. The cells were cultured in RPMI 1640 containing 10% FCS, antibiotics, and glutamine. Cells were used at confluence at the third to fifth passage. Coculture experiments were performed as described previously [Riemann et al., 1997]. Tissue culture plates were coated with fibronectin (10 μg/ml) in RPMI 10% FCS by incubation at 37°C for 1 h. For collagen I substrate, the plates were coated with a mix of 1 ml collagen I solution (3.56 mg/ml), 1 ml IST+ premix solution, and 18 μl 1 N NaOH for 1 h; excess solution was removed; and the plates were dried at room temperature.

RNA Isolation and Competitive RT-PCR

Total cellular RNA was isolated according to Chomczynski and Sacchi [1987]. Competitive reverse transcription-polymerase chain reaction (RT-PCR) for quantification of APN/CD13 mRNA expression was performed as previously described [Riemann et al., 1997]. Briefly, the first-strand DNA was synthesized using 0.5 μg total RNA in the presence of six dilutions of internal competitive standard RNA. Ten-percent portions of the cDNA were amplified with the following APN/CD13 specific primers: sense 5′GTCTACTGCAACGCTATCGC 3′; antisense 5′GATGGACACATGTGGGCACCTTG 3′. The relative amounts of target and standard products were calculated after densitometric analysis using ScanPack software 2.0.

Plasmid Construction

The pMyo construct, containing the myeloid promoter in front of the luciferase gene, was generated by inserting a 1.15-kb fragment (nucleotides −1158 to +65) of the human myeloid APN/CD13 promoter [Shapiro et al., 1991] as a SstI/SalI fragment into pGL3-basic digested with SstI/XhoI. A 300-bp CD13/APN enhancer fragment [enh; Olsen et al., 1997] was inserted as SalI/XhoI fragment into the SalI site downstream of the SV40 polyadenylation signal in pMyo to generate pMyo/enh. The pMyo/mut construct was generated by mutating the region −48 to −35 of the myeloid CD13/APN promoter using the GeneEditor system according to the manufacturer's instructions. To direct the mutation, an oligonucleotide 5′GAGTAGTGGTCGGG**AGCTCA**TACCTCCCTTTAAAAAG3′ carrying the mutation (bold) was used.

Preparation of Nuclear Extracts and DNase Footprinting

Nuclear extracts from T-cell lines as well as from colon carcinoma cells Caco 2 were prepared as described [Olsen et al., 1991; Troelsen et al., 1994]. The XbaI/SpeI digested fragment of the p-411luc construct was labeled on either strand with [γ-^{32}P] ATP, and the gel-purified fragments were used for DNaseI footprinting, which was performed as described previously [Olsen et al., 1991].

Gel-Shift Analysis

Gel-shift assay was carried out as described [Olsen et al., 1991]. The probe used was the promoter region found to be protected in the DNaseI footprinting with the following sequence:

5′ GTGGAGTAGTGGTCGGGTCCGGTTA-

CCTCCCTTT 3′

3′ CCTCATCACCAGCCCAGGCCAATGG-

AGGGAAATT 5′

Nuclear extract (5 mg) from HUT78 cells was incubated in a total volume of 9 ml EMSA buffer [5% (w/v) Ficoll 400, 25 mM Tris-HCl pH 7.9, 0.5 mM EDTA, 50 mM KCl, 5 mM $MgCl_2$, 110 mg/ml poly (dI-dC), and 11 mg/ml of sonicated salmon sperm DNA]. Samples were incubated for 10 min on ice, and the ^{32}P-endlabeled

double-stranded oligonucleotide was added (8 fmol). In some experiments, unlabeled double-stranded oligonucleotide was added in 100-fold excess. Supershift analyses were performed by adding appropriate antibodies (c-Myb, c-Ets1/2) followed by incubation at 16°C for 20 min. The labeled probe was added and the incubation continued for 15 min on ice. Finally, gel-shift loading buffer was added and samples were run on a 5% nondenaturing polyacrylamide gel. DNA-protein complexes were visualized by autoradiography. The relative intensity of obtained complexes was densitometric analyzed using the ScanPack 3.0 software.

Transient Transfection and Luciferase Assay

The human lymphoid leukemia cell line Jurkat was used for transient transfections because of much higher transfection efficiency compared to HUT78 cells. Using pEGFP and FACS analysis, we estimated the transfection efficiency for Jurkat cells ranged between 6% and 10%. Exponentially growing Jurkat cells were washed with OptiMem medium and resuspended in OptiMem medium at a concentration of 1×10^6 cells/ml. The appropriate plasmid (1.5 μg of firefly luciferase gene) and 0.15 μg of the internal control plasmid pRL-TK (*Renilla* luciferase gene) were cotransfected into the cells using DMRIE-C reagent according to the instructions of the manufacturer. The day after transfecting the cells, cocultivation of the transfected cells with SFCs or culture on ECM proteins (fibronectin, collagen I) was done for 4 h. Inhibitors were preincubated with lymphocytes for 30 min. Lymphocytes were harvested from SFCs as well as the ECM proteins by vigorous pipetting. The preparation of cell extracts and measurement of luciferase activities were carried out using the Dual-Luciferase reporter assay system according to the recommendations of the manufacturer. The assays for firefly luciferase activity and *Renilla* luciferase activity were performed sequentially using one reaction tube in a luminometer with two injectors. Changes in firefly luciferase activity were calculated and plotted after normalization with changes in *Renilla* luciferase activity in the same sample.

Statistical Analysis

Data are expressed as mean ± SD. The Wilcoxon rank sum test was used to determine whether two experimental values were significantly different, $P < 0.05$ indicated with *; $P < 0.01$ indicated with **.

RESULTS

Basal Expression of APN/CD13 mRNA in Lymphocytes

Using an RT-PCR assay with an internal competitive RNA standard, APN/CD13 mRNA expression was quantified in human tonsillar lymphocytes and in the human lymphoid leukemia cell lines HUT78 and Jurkat. In tonsillar T cells, the APN/CD13 mRNA expression was determined to be 3.8 ± 5.7 pg APN/CD13 mRNA/μg total RNA (n = 15). The T-cell line HUT78 expresses 4.3 ± 1.7 pg/μg total RNA (n = 5) and the Jurkat T-cell line expresses 0.2 ± 0.1 pg/μg total RNA (n = 5) and thus expresses APN/CD13 mRNA at a 20-fold lower level than HUT78 cells.

DNase I Footprint Analysis of the Myeloid Promoter

Nuclear proteins prepared from HUT78 cells and Jurkat cells were used to protect the myeloid APN/CD13 promoter (−411 to +65) against DNase I digestion. A single footprint (position −48 to −35) was observed with HUT78 with the following sequence: 5′ TCGGGTCCGGTTAC 3′ (Fig. 1). With nuclear extract of the T-cell line Jurkat, there was no clear footprint detectable.

Gel-Shift Analysis of the Proximal Promoter Element Protected in the Footprint Analysis

By means of gel-shift analysis, the binding of nuclear extracts of HUT78 cells to the sequence between −48 bp to −35 bp protected in the footprint analysis was verified. As shown in Figure 2, there is a DNA/protein complex detectable, which is competed out by using unlabeled specific oligonucleotide in excess. Addition of unlabeled oligonucleotides specific for transcription factor Sp1 did not compete the complex. Addition of an unlabeled oligonucleotide specific for the HNF1 transcription factor competed, however, weakly for binding. This is likely to be due to the presence of the sequence 5′ GGTTA 3′ in both the probe and the HNF1 binding site [Olsen et al., 1991], further confirming the assumption that this sequence is important for the binding of the unknown factor to the −48 to −35 region. With 5′ GTTA 3′

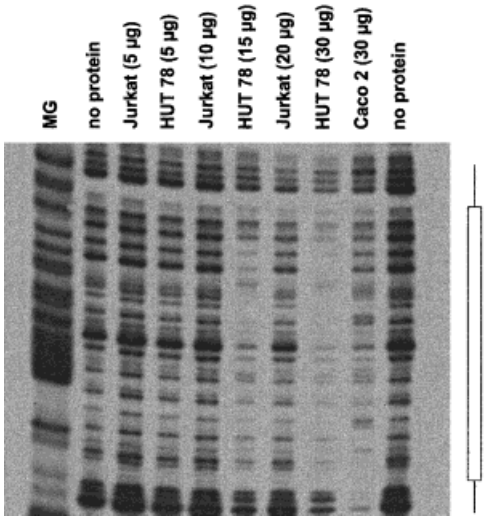


Fig. 1. DNase I footprinting of the aminopeptidase N (APN)/CD13 myeloid promoter. A DNA fragment (position −411 to +65 of the myeloid APN/CD13 promoter) was labeled, incubated without or with different concentrations of nuclear extracts obtained from T-cell lines Jurkat or HUT78, and digested with DNase I. The result of incubation with nuclear extracts of colon carcinoma cells Caco 2 is also shown. A Maxam Gilbert sequencing reaction is used as a marker (MG). Box marked indicates the protected region with the sequence 5′ TCGGGTC-CGGTTAC 3′.

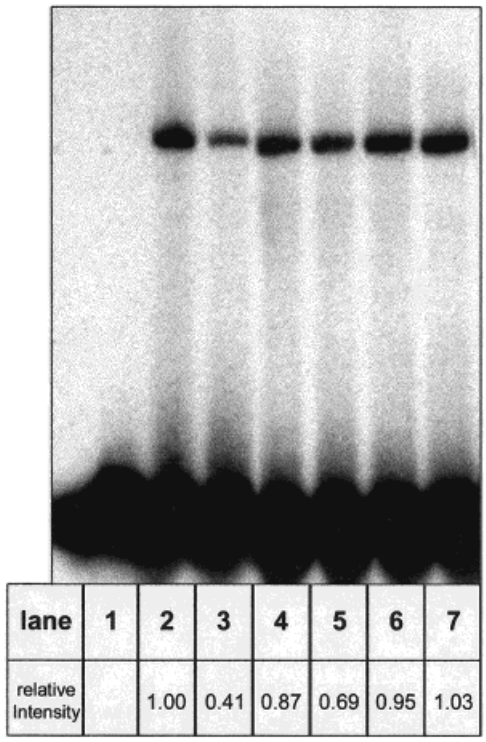


Fig. 2. Gel-shift analyses of the region protected in the footprinting. Gel-shift assay with nuclear extract (5 µg) of HUT78 cells was carried out with a labeled oligonucleotide (APN-Foot) covering the region protected in the footprinting of the aminopeptidase N (APN)/CD13 myeloid promoter (**lane 2**). Unlabeled APN-Foot oligonucleotide added to the reaction shows competition of the specific complex (**lane 3**). Addition of unlabeled oligonucleotides specific for transcription factors Sp1 does not compete the specific complex (**lane 4**). Addition of an unlabeled oligonucleotide specific for the HNF1 transcription factor competed weakly for binding (**lane 5**). Specific antibodies against c-Ets1/2 (**lane 6**) or c-Myb (**lane7**) added to the reaction do not supershift or compete the specific complex. **Lane 1** shows the labeled oligonucleotide without nuclear extract. The intensity of obtained complexes was densitometric analyzed and normalized to the intensity of complex in lane 2 (relative intensity = 1.0).

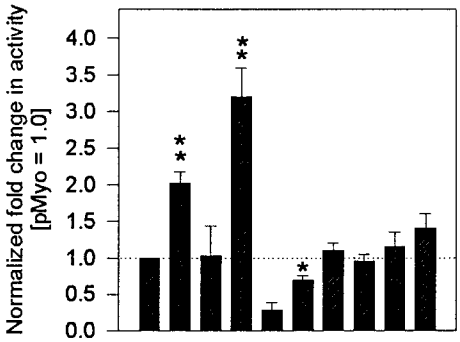
on the (+) and with 5′ GGA 3′ on the (−) strand, this footprint contains sequences possibly able to interact with the transcription factors Myb and Ets [Quandt et al., 1995]. The addition of specific antibodies against c-Myb as well as c-Ets1/2, however, did not result in a supershift of the DNA/protein complex or in a competition of the complex (Fig. 2).

Analysis of the Basal Promoter Activity in Lymphocytes

To further investigate the protected region detectable in the DNase I footprint analysis of the myeloid promoter, this region was mutated (pMyo/mut), transfected in Jurkat cells, and the reporter gene activity was quantified with the Dual-Luciferase assay system. Mutation of this region resulted in a significant decrease of the promoter activity by 71% (bar 5 in Fig. 3) compared to the activity of the nonmutated myeloid promoter (pMyo = 100%; bar 1 in Fig. 3), suggesting a functional role of this region in the basal APN/CD13 gene expression in lymphocytes. Furthermore, we tested the effect of the enhancer located between the myeloid and the epithelial APN/CD13 promoters. Addition of the enhancer to the myeloid promoter (the pMyo/enh construct) did not result in an increased basal activity of the promoter (1.1 ± 0.2-fold induction of the luciferase activity; bar 3 in Fig. 3).

Enhancer Involved in Cell-Cell Contact-Induced Expression of APN/CD13 Gene

Recently, we could show that exposure of tonsillar T and B cells, HUT78 cells, and Jurkat cells to SFCs results in a rapid increase of the steady-state level of APN/CD13 mRNA in the lymphocytic cells quantified by means of



	1	2	3	4	5	6	7	8	9	10
pMyo	+	+					+	+		
pMyo/enh			+	+					+	+
pMyo/mut					+	+				
SFC		+		+		+				
fibronectin							+		+	
collagen I								+		+

Fig. 3. Cell-cell contact induced aminopeptidase N (APN)/CD13 gene expression in lymphocytes. Jurkat cells were co-transfected with the appropriate promoter construct (pMyo, pMyo/enh, pMyo/mut) and the pRL-TK plasmid as described in Materials and Methods. When indicated, after 24 h transfected cells were allowed to adhere to fibroblast-like synoviocytes (SFC) or to extracellular matrix (ECM) for 4 h. Jurkat cells were harvested and lysed. Luciferase activity was measured using the Dual-Luciferase system, and relative changes in firefly luciferase activity were plotted after normalization to *Renilla* luciferase activity. The results are presented as average ± SD (n = 7). Asterisks indicate significant differences between transfected Jurkat cells after coculture on SFC as well as adherence on ECM and pMyo transfected cells as control, * $P < 0.05$; ** $P < 0.01$.

competitive RT-PCR [Riemann et al., 1997]. To characterize this increase, we measured the myeloid APN/CD13 promoter activity using transfected Jurkat cells cultured in the presence of SFCs for 4 h and quantified the reporter gene activity with the Dual Luciferase assay system. Jurkat cells transfected with the myeloid promoter construct (pMyo) showed a 2.0-fold increase of reporter gene activity after cell-cell contact with SFCs compared to cells without this contact (bars 1 and 2 in Fig. 3). The increased promoter activity was detectable already after 1 h of direct cell-cell contact of T cells with SFC (data not shown). Addition of the enhancer to the myeloid promoter (the pMyo/enh construct) further increased the response of the promoter to cell-cell contact (3.2-fold; bar 4 in Fig. 3). Contrary to this effect, adhesion of pMyo or pMyo/enh transfected Jurkat cells on fibronectin or collagen I did not increase the promoter activity (bars 7–10 in Fig.3). Mutation of the proximal promoter element defined by the DNaseI footprint analysis (the pMyo/mut construct) reduced the promoter response in Jurkat cells as described in the former paragraph (bar 5 in Fig. 3). The promoter activity in the presence of SFC (bar 6) was increased 2.3-fold ($P = 0.0079$) compared to activity with the mutant construct transfected in Jurkat cells (bar 5).

DISCUSSION

Peptidase induction via cell-cell contact seems to be a paradigm of peptidase regulation [Riemann et al., 1999], but regulatory mechanisms and signal transduction pathways involved still need more thorough investigation. In the study presented here we aimed at a better understanding of APN/CD13 expression on T lymphocytes after cell-cell contact with SFCs.

Our results demonstrate a basal expression of APN/CD13 mRNA in cells of lymphatic origin. We find an up to one thousand times lower expression in lymphocytes compared to the expressed transcript level in renal epithelial cells or in renal cell carcinoma cells [Kehlen et al., 1998a], in macrophages, or in myeloid cell lines [Shapiro et al., 1991]. Our absolute data for lymphocytic APN/CD13 mRNA expression agree with results by Wex and coworkers [Wex et al., 1995, 1997], who reported 6.5 ± 5.0 pg/μg total RNA for HUT78 cells.

The region from position −411 to −291 of the myeloid APN/CD13 promoter has previously been shown to contain binding sites for Myb and Ets transcription factors [Shapiro, 1995]. Moreover, by cotransfection of expression constructs for c-Myb and c-Ets1, a synergistic effect on the promoter was observed [Shapiro, 1995]. Furthermore, it was shown that the Myb-like DMP1 transcription factor binds to an Ets binding site and synergizes with c-Myb in activating APN/CD13 expression [Inoue et al., 1998]. We did not identify functionally important regions between −411 and −291 that are protected against DNase I digestion by HUT78 or Jurkat nuclear extracts. The present analysis, however, strongly suggests the presence of important regulatory sites in a narrow region (nucleotides −48 to −35) of the myeloid promoter. The presence of proteins binding to this region correlates with the level of APN/CD13 mRNA expression detectable in two T-cell lines. HUT78 cells, as cells with the

higher level of APN/CD13 mRNA, contain nuclear proteins binding to this region; whereas with proteins of the Jurkat cell line, no binding of nuclear proteins was observed. Jurkat cells express APN/CD13 mRNA at 20-fold lower levels compared to HUT78 cells or tonsillar T cells. Mutation of the protected region between −48 to −35 results in a strong decrease of the transcriptional activity probably due to inhibition of binding of stimulatory transcription factors. We describe for the first time a proximal promoter element in the myeloid promoter that is involved in the regulation of the *basal* lymphocytic APN/CD13 gene expression. The protected region between −48 to −35 with the sequence 5′ TCGGGTCCGGTTAC 3′ was analyzed to identify specific transcription factors binding to this region. With 5′ GTTA 3′ on the (+) and with 5′ GGA 3′ on the (−) strand, this footprint contains sequences, which may interact with the transcription factors Myb and Ets-1 [Quandt et al., 1995]. But using a gel-shift assay in combination with specific antibodies against Myb and Ets-1 could not verify this hypothesis.

Increased APN/CD13 mRNA and protein expression in lymphocytes could be shown after cell-cell contact of lymphocytes with SFCs, endothelial cells, epithelial cells, and monocytes/macrophages. Using competitive RT-PCR, we measured an up to 3.3-fold higher steady-state level of APN/CD13 mRNA in lymphocytes after cell-cell contact [Riemann et al., 1997]. In this work we have demonstrated that the activity of the myeloid promoter of the APN/CD13 gene is increased after cell-cell contact of lymphocytes with SFCs. Additionally, it is shown that an enhancer located between the myeloid and epithelial APN/CD13 promoter augments the cell-cell contact-induced expression of APN/CD13 on lymphocytes, suggesting an important role of this enhancer in the regulation of APN/CD13 induction after cell contact. Earlier we reported that the enhancer stimulated the transcription from a heterologous promoter (SV40 promoter) in myeloid cells [Olsen et al., 1997]. Here we show that the enhancer stimulates the activity of the myeloid promoter to result in an increase in lymphocytic APN/CD13 gene expression after cell-cell contact. The signals transducing this effect from contact sites to the nucleus remain to be clarified. Mutation of the region between −48 to −35, which is important for the *basal* transcription, seems not to influence the effect of cell-cell contact on the activity of the myeloid promoter because there is also with the pMyo/mut construct a more than twofold stimulation as was measured with the pMyo construct. We conclude that this region −48 to −35 is not important for the regulation of the lymphocytic APN/CD13 expression induced by cell-cell contact. Our finding of an increased lymphocytic APN/CD13 promoter activity upon interaction of lymphocytes with SFCs is in our opinion not due to allostimulation between lymphocytes and SFCs and subsequent T-cell receptor-mediated signaling. This conviction is based on our observation [Riemann et al., 1997] of a failing increased expression of marker molecules for activated T cells such as CD25 (IL-2 receptor α chain), CD69 (early activation molecule), or HLA-DR during cell-cell contact even of 2–3 days.

Adhesion of lymphocytes on ECM is not sufficient for increased APN/CD13 promoter activity. Previously we could show that contact of T cells with SFCs results in an activation of NF-κB and an increased IL-8 mRNA [Kehlen et al., 1998b] and protein expression in lymphocytes. Both effects, however, could also be observed after adhesion of lymphocytes on ECM proteins such as collagen I and fibronectin, a process that is not sufficient to upregulate lymphocytic APN/CD13 gene expression. Induction of APN/CD13 gene expression on the one hand, and activation of NF-κB and increase of IL-8 mRNA expression on the other hand, show a different stimulation pattern.

The interaction of lymphocytes with other types of cells is thought to be fundamental to many normal lymphocyte functions. Due to the interaction of lymphocytes with other cells, cellular functions could be altered, including the secretion of cytokines and growth factors [Piela and Korn, 1988; Scott et al., 1990; Bombara et al., 1993; Gombert et al., 1996; Burger et al., 1998]. Our finding that interaction of lymphocytes with SFCs can increase APN/CD13 promoter activity of T cells may have physiologic significance. The induced APN/CD13 protein expression represents a potentially increased cellular ability to inactivate inflammatory mediators, as shown for enkephalins or the chemokine MCP-1 [Amoscato et al., 1993; Weber et al., 1995]. Because APN/CD13 has been implicated in the trimming of peptides that protrude out of MHC class II molecules [Larsen et

al., 1996; Dong et al., 2000], CD13+ HLA-DR+ synovial T cells of people with rheumatoid arthritis could process and present special disease-related antigens. Moreover, APN/CD13 functions as a receptor for corona viruses [Delmas et al., 1992] and cytomegaloviruses [Giugni et al., 1996]. Therefore, the expression of APN/CD13 on lymphocytes may increase their susceptibility to pathogenic viruses. Further characterization of the induced APN/CD13 gene expression in lymphocytes after direct cell-cell contact with SFCs should provide ways to clarify the physiologic function of this molecule on lymphocytes.

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