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PGRL Is a Major CD81-Associated Protein on Lymphocytes and Distinguishes a New Family of Cell Surface Proteins

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CD81 exerts a range of interesting effects on T cells including early thymocyte differentiation, LFA-1 activation, and provision of costimulation. To better understand the mechanism by which CD81 influences T cell function we evaluated CD81 molecular complexes on T cells. The most prominent CD81-associated cellular surface protein on thymocytes as well as a number of T cell and B cell lines has an apparent molecular mass of 75 kDa. The 75-kDa protein was purified and analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry followed by postsource-decay profiling. p75 is a novel type I transmembrane protein of the Ig superfamily which is most similar to FPRP. We cloned and sequenced both human and mouse PG regulatory-like protein (PGRL) and characterized mouse PGRL expression in both lymphocytes and nonlymphoid tissues. The discovery of PGRL allows for the clustering of a small family of related proteins including PGRL, FPRP, V7/CD101, and IGSF3. Expression constructs containing various domains of PGRL with an epitope tag were coexpressed with CD81 and used to determine that the interaction of CD81 with PGRL requires the membrane distal Ig3–Ig4 domains of PGRL. Although it remains to be determined whether PGRL possesses PG regulatory functions, transwell chamber experiments show that PGs and CD81 coordinately regulate T cell motility. The Journal of Immunology, 2001, 167: 5115–5121.

The protein CD81 is a member of the tetraspanin family of integral membrane proteins which are widely expressed in the body and are found in all metazoans (1, 2). We have previously shown that CD81 is expressed at high levels by human T cells at all stages of development and that anti-CD81 (5A6) treatment of human thymocytes or mature T cells activates the integrin LFA-1 (3, 4). We and others have also shown that cross-linking of CD81 with Abs can provide a costimulatory signal with CD3 on human thymocytes, T cell lines, and naive mouse T cells (4–6).

Ab to CD81 (2F7) blocks T cell development at the CD4+8− stage in fetal thymic organ culture (FTOC), suggesting that CD81 is essential for T cell maturation (7). Subsequent to that report, three groups generated CD81-deficient mice and found that T cell development and function appears to be normal, with two interesting exceptions (8–10). First, it seems CD81 is required for T cell development (7). Second, these mice have impaired Th2 responses. Concordantly, CD81−/− mice are less sensitive to allergen-induced airway hyperreactivity (AHR) (11).

Recently we developed two new mAbs to mouse CD81 and found that CD81 is expressed on mouse thymocytes and that it is specifically up-regulated at the CD4+8+ stage (12). CD81 is then down-regulated on CD4+8− or CD4−8+ thymocytes and is low to negative on peripheral T cells but may be up-regulated upon activation. Despite these and many other functionally interesting roles for CD81, the mechanism by which CD81 (or any tetraspanin, for that matter) exerts its function is currently unknown. A principal model for tetraspanin function involves lateral associations with other cell-surface proteins (1). For example, CD81 associates with CD19, which forms a complex with CD21 (CR2) on B cells (13).

CD81 has also gained recent attention for its activity as a receptor for the hepatitis C virus envelope protein E2 (14–17). Despite the clear demonstration that E2 binds CD81, it seems that additional interactions between the virus and the host cell are required for fusion and viral entry (18, 19). It is not known what other host cell proteins may facilitate hepatitis C virus entry.

To better understand the function of CD81 on T cells, we sought to identify CD81-associated molecules. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry followed by postsource-decay (PSD) analysis and National Center for Biotechnology Information database queries were used to identify the major CD81-associated protein here named PG regulatory-like protein (PGRL). PGRL is a novel, 75-kDa, Ig superfamily member which is most similar to PG regulatory-like protein (PGRL). PGRL has also gained recent attention for its activity as a receptor for the hepatitis C virus envelope protein E2 (14–17). Despite the clear demonstration that E2 binds CD81, it seems that additional interactions between the virus and the host cell are required for fusion and viral entry (18, 19). It is not known what other host cell proteins may facilitate hepatitis C virus entry.

Materials and Methods

Cell lines and thymocytes

All mouse cell lines were maintained in RPMI 1640 with 10% FBS (Bio-Whittaker, Walkersville, MD). C6VL medium was also supplemented with 50 μM of 2-ME (Sigma-Aldrich, St. Louis, MO). COS-7 was maintained in DMEM with 10% FBS (Bio-Whittaker). Thymocytes were isolated from the thymi of BALB/c or C57BL/6 mice of various ages.

Antibodies

Abs used included EAT1 (anti-CD81); EAT2 (anti-CD81), previously described (12); 2F7 (anti-CD81) (Southern Biotechnology Associates, Birmingham, AL); and anti-V5 Ab (Invitrogen, San Diego, CA).

Biotinylation

Indicated cell lines were washed twice in cold PBS and resuspended in 5 ml of PBS supplemented with 2 mM of Mg2+ and 2 mM of Ca2+. EZ-Link N-hydroxysuccinimide long chain biotin (Pierce Endogen, St. Louis, MO) was added to a final concentration of 100 μg/ml. The cell suspensions were mixed and incubated on ice for 30 min. The cells were then washed in PBS supplemented with 2 mM of Mg2+ and 2 mM of Ca2+. After washing the cells were re-suspended in 500 μl of PBS with 2 mM of Mg2+ and 2 mM of Ca2+ to which 1 μl of EZ-Link N-hydroxysuccinimide long chain biotin (Pierce Endogen, St. Louis, MO) was added and the cell suspensions were incubated on ice for a further 30 min. The cell suspensions were washed with PBS, centrifuged at 200 × g for 5 min, and resuspended in 1 ml of PBS for flow cytometry analysis.
rockford, IL) was added at 1 mg/ml PBS and incubated at room temperature for 45 min. Cells were washed four times in cold PBS and lysed as described for immunoprecipitation.

**Immunoprecipitation**

Indicated cell types were lysed at a concentration of $5 \times 10^6$ cells/ml on ice for 1 h in TBS with EDTA (150 mM of NaCl, 5 mM of EDTA, 20 mM of Tris (pH 7.5)) with 1% of the indicated detergent (Triton X-100, IGEPAL CA-630 (Nonidet P-40), 3-[3-cholamidopropyl]-dimethylammonio-1-propanesulfonate (CHAPS), or Brij-97 (Sigma-Aldrich)) supplemented with 10 U/ml aprotinin (Calbiochem, San Diego, CA), 1 mM of PMSF, and 1 $\mu$g/ml pepstatin A. Lysates were clarified by centrifugation for 15 min at 15,000 rpm before use. Lysate was precleared for 1 h at 4°C against 10 $\mu$L of protein A-agarose (Pierce Endogen) or goat anti-mouse agarse beads (Sigma-Aldrich). Anti-CD81 Abs were captured on protein A-agarose (Pierce Endogen) at 5 $\mu$L of mAb/7.5 $\mu$L of beads, while 2 $\mu$L of anti-V5 Ab was captured on 7.5 $\mu$L of goat anti-mouse agarse (Sigma-Aldrich). Ab was preincubated with the indicated beads before addition of 2 $\times 10^5$ cell equivalents. Samples were rotated overnight at 4°C. Immunoprecipitated material was washed four times in lysis buffer, separated on SDS-PAGE, and transferred to polyvinylidene difluoride.

**Western blot**

Biotinylated material was blotted with streptavidin-HRP (1:2,500) (Southern Biotechnology Associates). EAT2 was used at 0.5 $\mu$g/ml. Biotinylated material was preincubated with the indicated beads before addition of 2 $\times 10^5$ cell equivalents. Lysate was run over a column of EAT1 Ab covalently cross-linked to protein A-agarose beads (Pierce Endogen) at 5 $\mu$L of goat anti-mouse agarose (Pierce Endogen) or goat anti-mouse agarse beads (Sigma-Aldrich). Anti-CD81 Abs were captured on protein A-agarose (Pierce Endogen) at 5 $\mu$L of mAb/7.5 $\mu$L of beads, while 2 $\mu$L of anti-V5 Ab was captured on 7.5 $\mu$L of goat anti-mouse agarse (Sigma-Aldrich). Ab was preincubated with the indicated beads before addition of 2 $\times 10^5$ cell equivalents. Samples were rotated overnight at 4°C. Immunoprecipitated material was washed four times in lysis buffer, separated on SDS-PAGE, and transferred to polyvinylidene difluoride.

**Cloning and construction of mouse PGRL constructs**

Total RNA was isolated from indicated mouse cell lines using RNeasy Mini kit (Qiagen, Valencia, CA) and was reverse transcribed using a Re- verse Transcription System (Promega, Madison, WI). PGRL was amplified from each cell line using mouse primers PGRL-3 (5'-CCAGGCGAAC GCGAACCCTTAGAA) and PGRL-2 (5'-CCGCATCCTTCTTCAAAAG). These primers begin amplification at the second Ig domain through to the stop codon and yield a 774-bp product. Primers for CD81 (5'-ATGGGAGTG GAGGGCTGCAC and 5'-AGTACACGGAGCTGTTCCGG) were used as a positive control.

**RT-PCR**

The Plg-2 construct PCR product from amplification of IMAGE clone 2649196 was used as the cDNA probe. The probe was [$32P$]-labeled using the Rediprime II random primer labeling system (Amersham Pharmacia Biotech, Little Chalfont, U.K.) according to the manufacturer’s instructions. The probe was hybridized to the MTN Blot according to the manufacturer’s instructions (Chontech Laboratories, Palo Alto, CA). Briefly, the denatured probe was incubated in ExpressHyb Hybridization solution at 68°C for 1 h, rinsed twice, and then washed for 30 min in 2X SSC plus 0.05% SDS at room temperature. The blot was further washed twice in 0.1X SSC plus 0.1% SDS for 40 min at 50°C before exposure for 18 h to BioMax MS film (Eastman Kodak, Rochester, NY).

**Construction of CD81**

C6VL cells (8 $\times 10^6$) were lysed in 1% Brij-97. Lysate was run over a column of EAT1 Ab covalently cross-linked to protein A-agarose beads (Pierce Endogen). Bound protein was eluted in 100 mM of glycine (pH 2.8), dialyzed, concentrated, and resolved on 16% Tris-glycine gradient gel (Invitrogen). Proteins were visualized with Coomassie blue and p75 was excised and subjected to trypsinolysis. MALDI-TOF mass spectrometry followed by PSD analysis was used to identify the identity of p75 (J. Leszyk, University of Massachusetts Medical Center, Worcester, MA).

**Protein purification**

C6VL cells (8 $\times 10^6$) were lysed in 1% Brij-97. Lysate was run over a column of EAT1 Ab covalently cross-linked to protein A-agarose beads (Pierce Endogen). Bound protein was eluted in 100 mM of glycine (pH 2.8), dialyzed, concentrated, and resolved on 16% Tris-glycine gradient gel (Invitrogen). Proteins were visualized with Coomassie blue and p75 was excised and subjected to trypsinolysis. MALDI-TOF mass spectrometry followed by PSD analysis was used to identify the identity of p75 (J. Leszyk, University of Massachusetts Medical Center, Worcester, MA).

**Cloning and construction of mouse PGRL constructs**

IMAGE clones 330850, 2649196, and 3593592 (Research Genetics, Huntsville, AL) were obtained and independently sequenced to verify PGRL sequence. All Plg constructs are V5-tagged PGRL proteins that were amplified from mouse IMAGE clone 2649196. Plg-1 contains only the membrane proximal Ig domain (missing amino acids 1–450); Plg-2 has the two membrane proximal Ig domains (missing amino acids 1–319); and Plg-4 represents the complete PGRL construct, missing only the putative secretion tag (amino acids 1–38) and with a mutation in the stop codon. Plg-1 was amplified using forward primer PGRL-3 (5'-GGCACTGTGTACC GGGGA) and reverse primer PGRL-2 (5'-CCGCATCCTTCTTCAAAAG). Plg-2 was amplified using forward primer PGRL-3 (5'-CCAC GCCGAACCTTGAAG) and PGRL-2; and Plg-4 was amplified using forward primer PGRL-1 (5'-CTTTACCGGGTGGCTGGCACC) and PGRL-2. Purified PCR products were cloned into pcDNA3.1/V5-His TOPO TA (Invitrogen). Inserts were cut out of pcDNA3.1/V5-His TOPO TA using Pmel and HindIII (Promega), which removes the insert along with the V5-His tag sequence. Digestion of pSecTag2 (Invitrogen) vector with Pmel and HindIII (Promega) removes the mycHis sequences. The fragments were then purified, ligated into the modified pSecTag2, and verified by sequencing.

**Northern blot**

Immunoprecipitated material was washed four times in lysis buffer, separated on SDS-PAGE, and transferred to polyvinylidene difluoride.
Cell migration experiments

SPI-4 cells were pretreated with 1 μg of the indicated PG in 1 ml (2.8 μM) of RPMI 1640 with 10% FBS (BioWhittaker) at 37°C overnight. Pretreated cells (6×10^5) were transferred to an 8-μm pore cell culture insert (BD Biosciences, Franklin Lakes, NJ) in serum-free RPMI 1640 supplemented with indicated Abs at 1 μg/ml. After a 4-h incubation, cells that had migrated through the membrane and fallen to the bottom of the well were counted with an Eclipse TE300 microscope (Nikon, Melville, NY). Automated cell counts were done with a Chemi-Imager using AlphaEase software, both from Alpha-Innotech (San Leandro, CA), with values indicated as an average of three×10 fields.

Results

CD81 molecular complexes on T cells

To identify CD81-associated molecules on T cells, T cell lines C6VL and TK-1 or thymocytes were biotinylated before lysis in different detergents. CD81 was immunoprecipitated with the hamster anti-mouse CD81 mAb EAT2 and associated molecules were visualized by Western blot with streptavidin-HRP (Fig. 1). As noted by others, the detergent plays a critical role in determining which and how many proteins remain associated with CD81 (20, 21). A number of prior reports have used CHAPS lysates to demonstrate tetraspanin associations; however, large complexes of proteins are not dissociated by CHAPS, perhaps remaining in micelles or detergent-resistant domains of the membrane. Notably, in Brij-97 lysates the major CD81-associated cell-surface protein on T cells has an apparent molecular mass of 75 kDa. The association with p75 is largely disrupted by Nonidet P-40 or Triton X-100. Consistent with previous experience, CD81 is poorly labeled with biotin in some cell lines such as MBL-2, PRL-9, CH27, and MS (Fig. 1C and Fig. 4A). The reason for differential reactivity of N-hydroxysuccinimide long chain biotin with CD81 is not known but may involve differences in CD81 conformation or associated proteins which influence access to the labeled amine group. It should be noted that all of the T cell lines shown in Fig. 1 express similar levels (median fluorescence intensity ~ 100) of CD81 as determined by flow cytometry. In addition to coimmunoprecipitation with EAT2, p75 was coprecipitated with 2F7 (Fig. 1D) and with EAT1 (not shown). Similar experiments were conducted on human T cells (Jurkat, HSB-2, and HPB-ALL) and gave a similar result (not shown). Anti-human CD81 (5A6) was used to immunoprecipitate biotinylated human T cell lysates, and Western blot with streptavidin shows a band at 75 kDa.

Identification of CD81-associated p75

To determine the identity of p75, 8×10^6 C6VL cells were lysed in Brij-97, run over a column of anti-CD81 (EAT1)-conjugated protein A-agarose beads, eluted in 100 mM of glycine (pH 2.8), dialyzed, concentrated, and resolved on 8–16% gradient SDS-PAGE and visualized with Coomassie blue. The p75 band was excised and subjected to trypsinolysis followed by MALDI-TOF mass spectrometry and PSD analysis. Protein Prospector software (Freeware from University of California, San Francisco, CA) was used to query various National Center for Biotechnology Information databases to determine the identity of p75. Four sequences derived from p75 (KDSQFSYAVFGPRV, RVLPDELQVSAAPGPGR, HAAYSVDWEPAMAPPGPGR, and LVAQL DTGIGSLPGYGEDRH) matched unpublished expressed sequence tags (ESTs) from mouse which cluster under UniGene ID number Mm.29860. These peptides were underlined in Fig. 2. The entire purification and MALDI-TOF mass spectrometry analysis was repeated and gave the same result. The peptide sequences were used to identify three IMAGE clones (33085, 2649196, and 3593592) which were obtained from Research Genetics and independently sequenced. Our sequences were then assembled with 85 ESTs using Vector NTI to determine the final mouse PGRL sequence which we have entered in GenBank under GI#2411055 (Fig. 2). The 75-kDa protein, PGRL, is a novel Ig superfamily member possessing an N-terminal secretion signal sequence shown in italics in Fig. 2; one transmembrane domain, which is bracketed in Fig. 2; and four Ig domains, which are bracketed in Fig. 3. PGRL contains three N-linked glycosylation sites immediately following the first conserved cysteines of the Ig-4, Ig-2, and Ig-1 domains, which are underlined in Fig. 3. Basic local alignment search tool analysis of the mouse PGRL sequence against the genome database revealed no significant matches.
human EST database yielded 152 ESTs which cluster under UniGene ID Hs.332012. The human PGRL was found in the human genome database and maps to 1q23.1 (physical position 16,663K). Interestingly, the human PGRL gene is not annotated. The human PGRL has five exons and possesses 90% identity with mouse PGRL at the amino acid level, excluding the secretion tag (Fig. 2).

**Distinction of the PGRL family**

Basic local alignment search tool analysis of the human genome database and the nr GenBank database with PGRL sequence reveals that there are three other genes closely related to PGRL. The similarity scores of PGRL, V7/CD101, IGSF3, and FPRP create a distinct cluster in which any of the four proteins used as a query will return the other three with an E value $e^{-70}$. No other known proteins, including other Ig superfamily members, have significant similarity to PGRL; therefore, this set of four genes may represent a small family. The amino acid alignment of the extracellular domain of PGRL with V7/CD101, FPRP, and IGSF3, each beginning at its N terminus, is shown in Fig. 3. V7/CD101, FPRP, and IGSF3 sequences contain additional Ig domains which have been truncated for the sake of space. V7/CD101 is expressed on T cells and was discovered as an inhibitor of T cell activation via the TCR (22). Nothing has yet been published regarding IGSF3. The protein most similar to PGRL is FPRP, with 28% identity (44% similarity) to PGRL. More specifically, Ig-4 and Ig-3 of PGRL align to the outer two Ig domains of FPRP with 33% identity (50% similarity). FPRP was discovered for its capacity to associate with the PGF$_2$ receptor and dampen its affinity for PGF$_2$ (23). FPRP was recently identified by two groups as the major CD81/CD9-associated

![FIGURE 3](http://www.jimmunol.org/)

**FIGURE 3.** Alignment of PGRL family members. The extracellular domain of human (h)PGRL (residues 1–575) is aligned with the N-terminal regions containing the first four Ig domains of hFPRP, hCD101, and hIGSF3. Ig domains are indicated in brackets. Underlined residues in hPGRL sequence indicate putative N-glycosylation sites. Regions of identity are highlighted in yellow, conserved residues are shaded gray, and green letters indicate weak similarity.
protein in nonlymphoid cells (20, 21). Both of these reports mark the band at 75 kDa as an unknown protein.

Expression and characterization of PGRL in non-T cells

PGRL association with CD81 was evaluated in the B cell lines A20, CH27, and MS. Cells were biotinylated, lysed in Brij-97, immunoprecipitated with EAT2, and Western blotted with streptavidin (Fig. 4A). As described above, CD81 is differentially labeled with biotin. Interestingly, MS is negative for p75 and is also negative for PGRL by RT-PCR (Fig. 4C). The identity of the prominent CD81-associated protein in MS is unknown, but its molecular mass of 116 kDa approximates that of FPRP.

PGRL expression in nonlymphoid organs (except spleen) was evaluated by Northern blot. Multitissue Northern blots were obtained from Clontech Laboratories and probed with 32P-labeled PGRL cDNA. Each lane contains 2 µg of polyA+ purified RNA, which has been confirmed for equal loading by a consistent signal for a housekeeping gene across all lanes. As predicted from the broad distribution of PGRL ESTs, PGRL is expressed in many tissues but exhibits strongest expression in the brain (Fig. 4B). PGRL appears to have a single major transcript of 2.5 kb.

Mapping of the CD81-PGRL association

To further characterize the interaction of CD81 with PGRL we constructed a series of PGRL expression vectors containing either the full extracellular domain (PIg-4), or Ig-2 and Ig-1 (PIg-2), or Ig-1 domain alone (PIg-1). Each of these constructs possesses the native PGRL transmembrane domains and the 6-aa cytoplasmic tail fused to the V5 epitope tag. Each construct, including PIg-4, uses the IgG secretion signal derived from the pSecTag2 vector rather than the native PGRL secretion tag to direct the molecule to the cell membrane. Each construct was completely sequenced and found to match the correct PGRL sequence. To determine that the V5-tagged PGRL proteins are produced as expected we transiently transfected 3T3 fibroblasts with each vector, cultured 2 days, and lysed before immunoprecipitation with anti-V5 followed by anti-V5 Western blot (Fig. 5A). Each vector produced V5-tagged PGRL of the expected sizes and in similar amounts. Interestingly, each PGRL construct also showed a minor band of roughly double the expected size, such as might be found with dimerization of the PGRL-V5 product. This larger product was also seen in Plg-4 at ~150 kDa but did not enter the gel shown. The small band at 35 kDa in Plg-4 may be a degradation product.

To evaluate which of the PGRL-V5 constructs interacts with CD81, 3T3 cells were transiently transfected, immunoprecipitated with anti-V5, and Western blotted for CD81. CD81 is coimmunoprecipitated with Plg-4 (Fig. 5B, upper panel). As a positive control, CD81 was immunoprecipitated from the same lysates and blotted for CD81 (Fig. 5B, lower panel).

To further confirm the interaction of CD81 and PGRL-V5, and to reduce the possible contribution of other proteins to this interaction, we transiently transfected COS-7 cells and conducted the same experiment. Because COS-7 is derived from monkey, it was necessary to cotransfect mouse CD81 in these experiments. We created a mouse CD81 expression construct in which full-length mouse CD81 is fused to a myc tag. This vector was completely sequenced and matched mouse CD81. As we found in 3T3 cells, CD81 coimmunoprecipitates with PGRL containing all four Ig domains but not with the truncated products lacking Ig-3 and Ig-4 (Fig. 5C). The fact that this coimmunoprecipitation works in monkey cells suggests that either the interaction is direct or a monkey protein is able to mediate the interaction.

T cell motility is modulated by PGs and CD81

The similarity of PGRL to FPRP suggests that one natural avenue of investigation would be to study whether CD81 and/or PGRL are physically or functionally related to PG receptor activities. While the full investigation of this possibility remains the subject of a

**FIGURE 4.** PGRL expression on mouse tissues and cell lines. Mouse B cell lines A20, CH27, and MS were biotinylated, lysed in Brij-97, and immunoprecipitated with EAT2. A. Associated molecules were visualized by Western blot with streptavidin-HRP. B. Northern blot analysis of PGRL expression using 32P-labeled Plg-2 cDNA as a probe. C. RT-PCR using primers PGRL-2 and PGRL-3 was performed on mouse T and B cell lines to evaluate PGRL expression. CD81 expression is shown as positive control.
FPRP was recently identified by two groups as a major CD81/PG receptor (not shown). The PGRL sequence is most similar to FPRP, which member, which we have named PGRL, as a major CD81-associated protein. The PGRL sequence is most similar to FPRP, which is a negative regulator of the PG receptor PGF2α. Interestingly, FPRP was recently identified by two groups as a major CD81-associated protein on nonlymphoid cells. Based on their sequence similarity and the finding that both FPRP and PGRL share an association with CD81, it seems reasonable to consider the possibility that PGRL may possess a function similar to FPRP.

It is worth noting that there are many parallels between the role of PGs and the role of CD81 in T cell development and function. PGs are a pervasive class of inflammatory mediators produced by the action of cyclooxygenases (COX-1 or COX-2) on arachidonic acid. PGE2 is particularly abundant in the thymus, where its production is differentially regulated by cortical and medullary epithelial cells and dendritic cells. Mouse thymi depleted of hematopoietic cells by 2-deoxyguanosine, such as in FTOC, produce PGE2 and PGF2α (27). Interestingly, COX-1-dependent synthesis of PGE2 acting via the EP2 receptor, facilitates differentiation of T cells from the CD4+ stage to the CD4+8+ stage (28). Pharmacologic inhibition of PGE2 synthesis impairs the adhesion of CD4+8+ thymocytes to thymic stromal cells, which is rescued by exogenous addition of PGE2 (26). It is possible that these effects are related to the fact that CD81 is specifically up-regulated on CD4+8+ cells during T cell differentiation and that anti-CD81 (2F7) is reported to prevent the development of CD4+8+ cells in FTOC.

In the periphery, PGE2, which is produced by macrophages, dendritic cells, and B cells, exerts a suppressive effect on Th1 future study (and awaits production of an anti-PGRL Ab) we have found encouraging results suggesting that CD81 and PG signals may be functionally connected.

There are five classes of PG receptors, designated EP, FP, IP, TP, and DP, and within the EP category there are four receptors that account for the total of eight known PG receptors (24). T cells express EP3, EP4, IP, and TP (25). The functional roles of PG receptors on T cells are given further attention in Discussion. Surprisingly, very little has been reported concerning the effect of these inflammatory mediators on T cell adhesion and motility. Both PGE1 and PGE2 bind to EP3 and EP4 with similar affinities (25). We tested the effect of PGE1 or PGE2 on T cell motility in the presence or absence of anti-CD81 mAbs 2F7 or EAT1. Interestingly, EAT1 but not 2F7 in the presence of PGE1 or PGE2 induces motility of SPI-4 mouse T cells (Fig. 6). EAT1, 2F7, or PGs alone do not influence T cell motility. Similar to EAT1, EAT2 also promotes T cell motility (not shown). Exposure of T cells to PGs for 15 min or 24 h does not change the level of CD81 expression on T cells as assessed by flow cytometry (not shown). Additionally, PG treatment does not change the pattern of CD81-associated proteins as determined by biotinylation-immunoprecipitation experiments (not shown).

The new mAbs to mouse CD81, EAT1, and EAT2 activate the integrin LFA-1 on T cells (our unpublished data). This finding parallels our work with 5A6 on human T cells (3, 4). To test whether specific PGs influence the ability of CD81 to activate LFA-1 we used a cell-cell adhesion assay. EAT2 promotes LFA-1 mediated T cell-T cell adhesion, which is inhibited by coincubation with PGE1 or PGE2 but not by PGB2 or PGF2α (not shown). None of the PGs tested independently promotes cell-cell adhesion, nor does another Ab to mouse CD81 (2F7) induce LFA-1-mediated adhesion in the presence or absence of PGs (not shown).

Discussion

One model for CD81 function is that it may serve as a transmembrane adapter protein which mediates the assembly of signaling receptor molecules. In this report we identify an Ig superfamily member, which we have named PGRL, as a major CD81-associated protein. The PGRL sequence is most similar to FPRP, which is a negative regulator of the PG receptor PGF2α. Interestingly, FPRP was recently identified by two groups as a major CD81/CD9-associated protein on nonlymphoid cells. Based on their sequence similarity and the finding that both FPRP and PGRL share an association with CD81, it seems reasonable to consider the possibility that PGRL may possess a function similar to FPRP.
responses and augments Th2 responses. PGE₂ suppresses IL-2 and IFN-γ but not IL-4 or IL-5 (29). PGE₂ up-regulates macrophage-derived chemokine production, which selectively recruits Th2 cells while suppressing IFN-inducible protein-10, which selectively recruits Th1 cells. Macrophage-derived chemokine production may be important for recruitment of Th2 cells during allergic airway inflammation (30). Additionally, PGE₂ promotes differentiation and synergistically enhances IL-4 and LPS-driven B cell class-switching to IgE (31). Interestingly, PGD₂ receptor-deficient mice exhibit greatly reduced levels of infiltrating lymphocytes and Th2 cytokines compared with normal mice in an allergic asthma model (32). It is possible that there is a connection between these findings and studies showing that CD81+/− mice have impaired Th2 responses and are resistant to AHR.

Regarding the physical nature of the PGRL-CD81 association we consider it likely that the two are directly associated. Lymphocytes generally do not express FPRP (D. Orlicky, personal communication, and our unpublished data) and therefore the association of PGRL with CD81 is not mediated by FPRP. Similarly, the mouse T cell lines used in our study do not express CD9, which excludes CD9 as a mediator of the PGRL-CD81 interaction, unlike the apparent role of CD9 as a mediator of the association between CD81 and FPRP (20). Additionally, the finding that PGRL associates with CD81 when transfected into COS-7 suggests that the interaction is direct. Future studies will determine whether a PG receptor is physically associated with PGRL and/or CD81. It will also be important to determine whether PGRL and/or CD81 influence the specific binding of a PG or the activities of a PG receptor. The specific PG receptor remains to be established, but the antagonistic effect of PGE₁ and PGE₂ on CD81-induced LFA-1 activation and the ability of PGE₂ to facilitate T cell motility in the presence of anti-CD81 hint that PGRL may regulate PGE₂ responses. This hypothesis is particularly attractive given the parallels of PGE₂ and CD81 in AHR and T cell development.

References