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Ecto-enzyme and signaling functions of lymphocyte CD73

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Summary: CD73 or ecto-5'-nucleotidase (5'-NT) is a widely expressed ecto-enzyme which catalyzes the dephosphorylation of AMP and other nucleoside monophosphates. CD73 participates in purine salvage through this enzymatic activity, supplying cells with precursors for energy metabolism and nucleic acid biosynthesis. As an enzyme that produces adenosine, CD73 can also regulate adenosine receptor engagement in many tissues. However, CD73 also has functions independent of its enzyme activity. Like many glycosyl phosphatidylinositol (GPI)-anchored molecules, it transmits potent activation signals in T cells when ligated by antibodies. Less compelling evidence suggests that CD73 may function as a cell adhesion molecule. In the human immune system, CD73 is expressed on subsets of T and B cells, on germinal center follicular dendritic cells, and on thymic medullary reticular fibroblasts and epithelial cells. Many challenging areas remain to be explored before the role of CD73 in the immune system will be fully understood. These include an evaluation of the role of adenosine receptors in lymphoid development, the identification of physiological CD73 ligands, a functional assessment of the GPI anchor, and an analysis of the intricate cell-type-specific and developmental regulation of CD73 expression.

Introduction

CD73, or ecto-5'-nucleotidase (5'-NT, EC 3.1.3.5), is a widely expressed ecto-enzyme that catalyzes the dephosphorylation of purine and pyrimidine ribo- and deoxyribonucleoside monophosphates to the corresponding nucleosides. It hydrolyzes only 5'-, and not 2'- or 3'-, monophosphates. While the enzyme has a broad substrate specificity, it prefers purine ribonucleoside monophosphates, and 5'-AMP is the best substrate, with K_M values of 3–6 μM (1, 2). Although 5'-NT is found in most tissues, its expression is usually restricted to specific cell types. In the human immune system, for example, CD73 is expressed on subsets of T and B cells, on germinal center follicular dendritic cells (FDC) (3), and on reticular fibroblasts and epithelial cells of the thymic medulla*. There are several examples where 5'-NT and adenosine deaminase (ADA), the next enzyme in the purine

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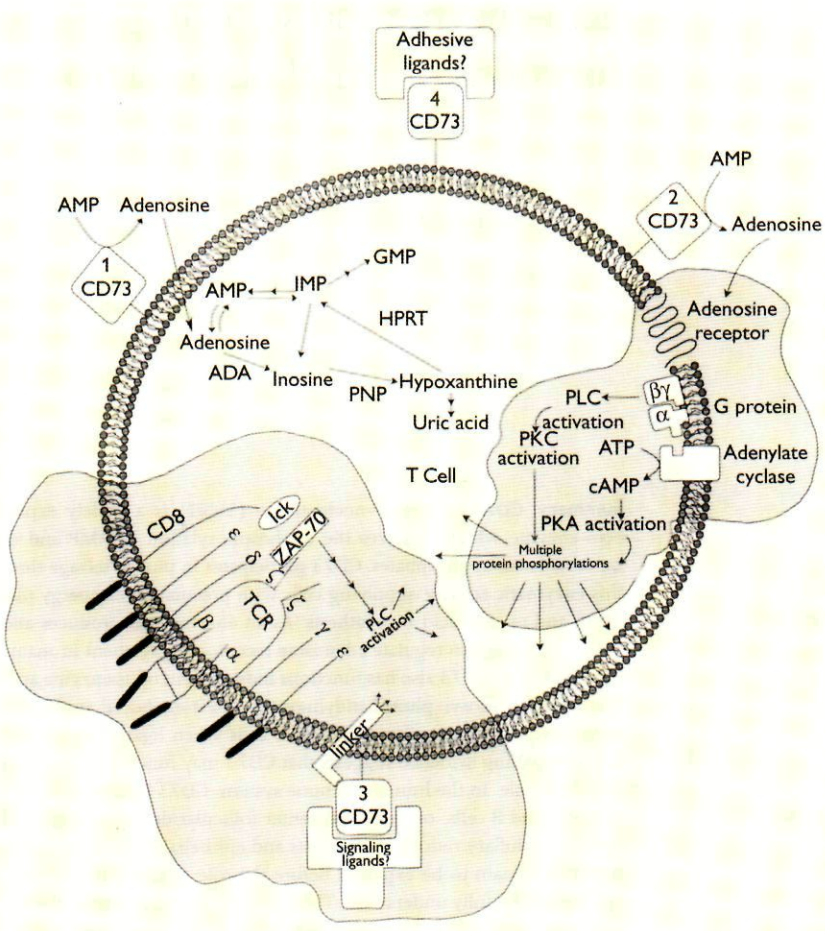

Fig. 1. CD73 is a multifunctional ecto-enzyme. Four proposed functions for CD73 are shown and numbered in the order in which they are discussed in the text. 1. Purine salvage, 2. Synthesis of adenosine for adenosine receptor signaling, 3. Co-stimulation of T-cell activation mediated through the CD3/TCR complex, and 4. Cell adhesion.

salvage pathway, appear to be inversely regulated in a cell-type-specific fashion. Since 5'-NT produces adenosine and ADA degrades it (*Fig. 1*), these observations suggest an important role for both these enzymes in the control of extracellular adenosine levels. In fact, early interest in 5'-NT stemmed from the fact that it produces adenosine, which acts as a potent vasodilator by triggering adenosine receptors in the cardiovascular system. However, it is important to remember that 5'-NT has a broad substrate specificity and high specific activity giving it the capacity to contribute substantially to the salvage of both purines and pyrimidines in the cell types in which it is expressed. In addition to its functions as an enzyme, 5'-NT, like several glycosyl phosphatidylinositol (GPI)-anchored lymphocyte proteins, serves as a co-stimulatory molecule for the CD3/T-cell receptor (TCR) complex. Less compelling evidence suggests that 5'-NT may also play a role in cell adhesion. Since the molecular aspects of 5'-NT have been thoroughly reviewed relatively recently (4), this review will focus on the functions of the enzyme which may be of particular interest to immunologists. It will be apparent to the reader that there are important gaps in our knowledge of this multifunctional enzyme, revealing areas well-suited for further investigation.

Historical perspective: how CD73 came to the attention of immunologists

As early as the 1970s, it was recognized that human lymphocytes were heterogeneous with respect to 5'-NT activity, and that chronic lymphocytic leukemia (CLL) B cells could be either 5'-NT$^+$ or 5'-NT$^-$ based on a histochemical stain (5). B-CLL cells from the majority of patients examined had either low or even undetectable 5'-NT expression. Cord blood lymphocytes were also known to have only about 1/5 the 5'-NT enzyme activity of lymphocytes from adults (6). Given that CLL lymphocytes (7) and cord blood B lymphocytes (6) were believed to be immature, these observations suggested that the level of 5'-NT in B cells might correlate with maturity. Prior to 1977, however, 5'-NT was primarily known to immunologists

as a useful plasma membrane marker in subcellular fractionation experiments (8).

Later that year and in the following year, two publications heightened immunologic interest in this ecto-enzyme. Reduced 5'-NT enzyme activity was found in the peripheral blood mononuclear cells (PBMs) of patients with common variable immunodeficiency (CVI) (9) and X-linked agammaglobulinemia (XLA) (10). CVI is a heterogenous disease characterized by hypogammaglobulinemia and low to normal numbers of B lymphocytes (11). Most CVI patients have intrinsic B-cell defects. XLA patients also have hypogammaglobulinemia, but the cause is a failure of pre-B-cell growth and clonal expansion (12).

5'-NT is part of the same metabolic pathway that contains ADA and purine nucleoside phosphorylase (PNP) (Fig. 1). In the early 1970s, it had just been discovered that mutations in the genes encoding ADA and PNP led to severe combined immunodeficiency (SCID) (13) and an isolated T-cell immunodeficiency (14), respectively. Since an intact purine salvage pathway seemed necessary for normal lymphocyte development, it was at first postulated that CVI and XLA might be caused by mutations in the structural gene for 5'-NT that led to decreased enzyme activity. However, two pieces of evidence argued against this. First, the residual 5'-NT enzyme activity in CVI and XLA patients was 30–50% of control levels, much higher than was seen in ADA- or PNP-deficient patients who usually had only 1–3% or less of control enzyme levels. Second, total body purine metabolism was not abnormal in CVI and XLA patients (15), unlike ADA- and PNP-deficient patients who showed strikingly abnormal purine metabolites in serum, urine, and erythrocytes (16). Thus, 5'-NT deficiency in CVI and XLA patients appeared to be limited to lymphocytes.

To better understand the basis for the reduced 5'-NT enzyme activity in PBMs from these patients, purified populations of normal T and B cells were studied at different stages of maturation. 5'-NT enzyme activity was 5- to 6-fold higher in peripheral blood B cells than T cells (17, 18). Thus, much of the reduced 5'-NT enzyme activity in PBMs from patients with XLA was explained by their B-cell deficiency. Fetal spleen and cord blood B cells had only 1/5 to 1/6 the enzyme activity of adult blood B cells (19, 20). B-cell CD73 expression increased to adult levels during the first 6 months of life, over the same time period when infants' B cells gained the ability to make IgG antibody responses (21). Purified B cells from many CVI patients had low 5'-NT activity . This correlated with an inability to synthesize IgG *in vitro* and was, therefore, consistent with a block in maturation. Lymphocyte 5'-NT enzyme activity was also measured sequentially in 2 twin infants, one of whom exhibited transient hypogammaglobulinemia of infancy. The infant with normal B-cell development showed the normal increase in lymphocyte 5'-NT activity in the first few months of life, while the other twin had a delayed increase in 5'-NT activity of almost 2 years which correlated with her recovery from hypogammaglobulinemia (21) (L.F. Thompson & J.F. Bastian, unpublished observations). Taken together, these data suggest that 5'-NT activity increases during B-cell maturation.

The 5'-NT activity of thymocytes was only about 1/10 that of adult peripheral blood T cells (22), suggesting that the level of 5'-NT expression might also be an indication of T-cell maturity. We were surprised, therefore, to find reduced 5'-NT enzyme activity (10–50% of normal) in the T cells of patients with XLA and CVI (17, 23) since most patients with these diseases are believed to have normal T-cell function (24, 25). Further analysis revealed that this was caused by strikingly reduced percentages of 5'-NT$^+$ T cells, especially in the CD8$^+$ subset (7.5% vs. 35% for control subjects) (23, 26, 27). Low percentages of 5'-NT$^+$ T cells might be explained by the number of infections which hypogammaglobulinemic patients have experienced, given the later observation that memory CD8$^+$ T cells are largely 5'-NT$^-$ (28). Patients with infectious mononucleosis also had a profound, though transient, expansion of CD8$^+$ 5'-NT$^-$ T cells (29).

5'-NT enzyme activity was also measured in PBMs of patients with a wide variety of other immunodeficiency diseases, including Wiskott-Aldrich syndrome, DiGeorge's syndrome, selective IgA deficiency, SCID, Omenn's syndrome, ADA deficiency, and AIDS (15, 20, 29–36). 5'-NT enzyme activity in PBMs of patients was almost always significantly lower than that of age-matched controls and was absent in the handful of SCID patients studied. Assessment of 5'-NT enzyme activity was useful in a clinical setting since the results could be obtained in a single day as compared to mitogen studies which required 3 days.

Distribution of CD73 on lymphoid tissue by immunofluorescence

Observations of low 5'-NT enzyme activity in PBMs of patients with immunodeficiency diseases provided the original impetus for an evaluation of 5'-NT expression on normal lymphocytes at different stages of differentiation. These studies relied on enzyme assays and histochemical stains and were cumbersome because anti-5'-NT mAbs were not yet available. Nevertheless, it was found that 5'-NT expression on lymphocytes was developmentally regulated, suggesting that the enzyme might be important for lymphocyte maturation. Now, with the advent of

transgenic (37) and knockout technology (38), and the availability of mAbs to human (3, 39–44), mouse*, and rat 5'-NT (45), there are a variety of new model systems to explore the function of 5'-NT in the immune system. Often, insight into the function of a molecule can be obtained by comparing its pattern of expression in multiple species. Thus, we summarize below the tissue distribution of CD73 in human and mouse lymphoid tissue.

Human thymus

Our recent examination of CD73 expression in human thymus produced unexpected results*. In 1982, Ma et al. (46) reported 5'-NT enzyme activity in purified thymocyte subsets at various stages of differentiation. Medullary thymocytes possessed approximately 5-fold higher 5'-NT enzyme activity than cortical thymocytes, a level similar to that of peripheral T cells. These results were consistent with increased 5'-NT activity in thymuses of rodents treated with cortisone as compared to control animals (47, 48). In our studies, $CD4^+CD8^+$ thymocytes were $CD73^-$, as expected. However, CD73 was also absent in CD4-single positive cells and only 4–19% of CD8-single positive cells were $CD73^+$ (as compared to 50% for adult peripheral blood $CD8^+$ cells). The percentage of $CD73^+$ cells was higher in thymuses from 2 children aged 4 and 11 years than from 2 infants aged 1 week and 6 months. Immunohistochemical evaluation of CD73 expression in the thymus did show intense staining in the medulla, but it was associated primarily with reticular fibroblasts and epithelial cells rather than thymocytes. The most likely reason for the discrepancy in our results compared to those of Ma et al. is the lack of precision in the method they used to prepare medullary thymocytes. Similarly, the primary reason for the higher 5'-NT enzyme activity of thymuses from cortisone-treated animals is probably their increased percentages of epithelial cells compared to thymocytes. Since T-cell CD73 expression in cord blood is similar to that in adults, our results suggest that the level of CD73 expression increases substantially after T cells leave the thymus. If CD73 expression is important for normal thymic development, we propose that the CD73 on stromal/epithelial cells plays a dominant role.

Human peripheral blood T cells

In adult peripheral blood T cells, approximately 19% of $CD3^+$, 11% of $CD4^+$, and 51% of $CD8^+$ cells are positive for CD73. All 5'-NT^+ T cells are $CD28^+$ (3). When $CD8^+$ cells are divided into $CD11b^+$ and $CD11b^-$ subsets, the expression of 5'-NT is restricted to $CD11b^-$ cells (28, 49). In addition, CD73 is preferentially expressed on $CD45RA^{hi}$ $CD45RO^{lo}$ (naive) $CD8^+$ T cells as compared to memory T cells in both peripheral blood (28) and tonsil (50). $CD16^+$ NK cells are 5'-NT^- (39).

Human B cells

Approximately 3/4 of adult peripheral blood, spleen, and lymph node B cells express CD73 (3, 39). Recent studies by Airas document CD73 expression in tonsillar B cells (50). B cells in primary follicles are largely $CD73^+$, as are resting $sIgD^-CD38^-$ memory B cells. In contrast, $CD38^+$ germinal center B cells are $CD73^-$. Thus, as in T cells, CD73 expression is regulated at several stages of B-cell differentiation.

Murine CD73

Immunofluorescence studies with recently developed rat anti-murine CD73 mAbs* revealed both similarities and differences between CD73 expression on murine versus human leukocytes. Subpopulations of murine spleen and lymph node T cells expressed CD73 as was seen with human T cells. Furthermore, murine spleen T cells could be induced to proliferate and secrete IL-2 when cultured with anti-CD73 mAbs plus submitogenic concentrations of phorbol myristate acetate (PMA), suggesting that both murine and human CD73 transmit activation signals (see below). In B cells, however, the expression pattern was quite different. Most $CD19^+$ cells in the spleen were $CD73^-$ and there were no $CD73^+$ B cells in lymph nodes. Early studies by Barton & Goldschneider suggested that CD73 expression may also be low on rat B lymphocytes (51). $sIgA^+$ B cells in Peyer's patches were, however, $CD73^+$. Many murine bone marrow myeloid cells were $CD73^+$, while human myeloid cells were negative. CD73 expression was also found on murine bone marrow stromal cell lines and the thymic epithelial cell line TEC. Immunohistochemical staining confirmed the high expression of CD73 in non-lymphoid elements of the thymus, spleen, and lymph node.

The striking differences in CD73 expression between human and murine CD73 are thought-provoking regarding the proposed importance of CD73 in human B-cell development. Perhaps there is another cell-surface phosphatase which takes over the function of CD73 on murine B cells. Alternatively, another cell type may express CD73 in murine lymphoid tissue. Indeed, histochemical analysis of CD73 expression in murine spleen and lymph node demonstrated that the B cells in those tissues are surrounded by a reticular network which is highly $CD73^+$. The relative lack of antigen stimulation in a specific pathogen-free animal facility could also account for the low

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level of murine B-cell CD73 expression. Perhaps sIgA$^+$ B cells in Peyer's patches are CD73$^+$ because they have encountered antigen. Our observation that human B-cell CD73 expression increases during the first 6 months of life is consistent with this hypothesis (21).

The finding of CD73 expression on bone marrow stromal cells and thymic epithelial cells suggested that CD73 might mediate interactions between stromal elements and developing lymphoid precursors. However, CD73 mAbs had no effects on either Whitlock-Witte or fetal thymic organ cultures in terms of the number of cells produced or their phenotypes. These results do not preclude the possibility of such interactions, however, as the relevant epitopes may not be recognized by the available anti-CD73 mAbs. Alternatively, CD73 enzymatic activity may be an important factor and this is not completely inhibited by anti-CD73 mAbs. Thus, it will be important to evaluate the effect of the specific 5'-NT inhibitor α,β-methylene adenosine 5'-diphosphate (1) in these *in vitro* models.

Functional assays on 5'-NT$^+$ and 5'-NT$^-$ lymphocytes

T cells

The unequivocal demonstration of 5'-NT$^+$ and 5'-NT$^-$ lymphocytes prompted us to ask whether 5'-NT$^+$ lymphocytes could perform certain functions that 5'-NT$^-$ lymphocytes could not. Thus, we sorted purified preparations of T cells into 5'-NT$^+$ and 5'-NT$^-$ populations using our goat anti-5'-NT antibodies (52). Since it was known that 5'-NT enzyme activity was very low in most human thymocytes (22, 46), we considered the possibility that 5'-NT$^-$ peripheral blood T cells might be functionally immature. However, we found that CD73$^-$ T cells made proliferative responses to mitogen (PHA) and allogeneic cells which were equal to or better than those of CD73$^+$ T cells. CD73$^-$ T cells were also able to provide more help for pokeweed mitogen (PWM)-stimulated antibody production than CD73$^+$ T cells. This is probably because CD73$^-$ T cells contain a lower proportion of CD8$^+$ cells. In contrast, CD73$^+$ T cells responded to lower doses of PMA with greater increases in [^{3}H]-thymidine incorporation.

B cells

We also performed parallel functional studies on CD73$^+$ and CD73$^-$ B cells (53). In this case, our results clearly supported our hypothesis that CD73 expression was an indication of B-cell maturity. While both CD73$^+$ and CD73$^-$ B cells could synthesize equivalent amounts of IgM in response to *in vitro* stimulation with either PWM or Epstein-Barr virus (EBV), the ability to make IgG was restricted to the CD73$^+$ subset.

CD73 expression in lymphoid malignancies

CD73 is also expressed in some lymphomas and lymphoid leukemias. 5'-NT activity is low in the vast majority of patients with CLL (5), and it is not known whether the minority of patients with high CD73 expression have a different prognosis. In acute lymphoblastic leukemia (ALL), CD73 is expressed only among CD10$^+$ cases (54). Immature CD10$^-$ pro-B ALL, mature B ALL, and T-cell ALL express little or no CD73. However, not all CD10$^+$ cases are CD73$^+$. CD73 expression is associated with a poor clinical outcome in both children (55) and adults (56) with ALL. This poor outcome, however, is not related to resistance to the thiopurines 6-mercaptopurine and 6-thioguanine used to treat ALL (57). Ujházy et al. (58, 59) made the interesting observation that CD73 expression increased with the acquisition of the multidrug-resistant phenotype in murine lymphoma cells exposed to doxorubicin. When EL4 cells were grown in increasing concentrations of this chemotherapeutic agent, they acquired expression of the multidrug resistance protein P-170, as expected. Additionally, CD73 expression increased. In fact, inhibition of CD73 enzyme activity caused a reversion to doxorubicin sensitivity among the P-170$^+$ cell lines. Ujházy et al. hypothesized that 5'-NT enzyme activity was needed to salvage the ATP (after its conversion to AMP via other ecto-enzymes) which was lost when P-170 pumped doxorubicin out of the cell. The mechanism responsible for this intriguing association between P-170 and CD73 expression remains to be determined. It will also be important to determine whether CD73 expression increases *in vivo* in cancer patients with lymphoid malignancies as multidrug resistance is acquired.

CD73 structure, gene cloning, and regulation

CD73 has a GPI membrane anchor

CD73 could be released from placental plasma membranes (60) or from the surface of human lymphocytes (3) by incubation with highly purified phosphatidylinositol-specific phospholipase C (PI-PLC) from *B. thuringiensis*. This was strong evidence that at least a fraction of the enzyme was anchored into the membrane via a GPI anchor. Formal proof came when human CD73, released from placental plasma membranes by PI-PLC treatment, and further purified by affinity chromatography on Con A Sepharose and a mAb column, was subjected to acid hydrolysis and mass spectophotometric analysis for quantitation of inositol (61). Approximately 1 mole of inositol was found per mole of enzyme, confirming the existence of the GPI anchor. Misumi et al. (62) also identified components of the GPI anchor when they purified the carboxyl terminal pep-

tide of human placental 5'-NT. We also found 1 mole of inositol/mole of protein in a soluble form of CD73 purified from the 100,000 × g supernatant fraction of human placental extracts. This suggests that "soluble" CD73 had been previously linked to the membrane via a GPI anchor. Inositol was also detected in soluble 5'-NT purified from electric ray, suggesting that it, too, had been previously GPI-anchored (63). Not all CD73 is released from plasma membranes by PI-PLC, and mass spectrometric analysis of purified "PI-PLC-resistant" CD73 from human placenta showed 0.45 mole inositol/mole of enzyme. These data show that at least part of the PI-PLC-resistant CD73 also has a GPI anchor. The lack of equimolar concentrations of inositol and enzyme could be due to a transmembrane form of the protein or to impurities in the preparation analyzed. It is still not known whether an alternative form of CD73 exists with a conventional transmembrane anchor. However, cDNAs corresponding to only one isoform of CD73 have been cloned, and their sequences are consistent with the GPI-anchored form of the molecule (47, 62, 64–66). CD73, thus, belongs to a large group of surface proteins which are GPI-anchored, many of which have been implicated in cellular signaling (67, 68).

Cloning of CD73

5'-NT has been purified and characterized from a variety of sources, including porcine intestinal smooth muscle (69), human placenta (61, 70–72), human endothelium (73), rat heart (2), and electric ray electric organ (74). The cDNA was first cloned from rat liver and human placenta by Misumi et al. (62, 64). The human cDNA encodes 574 amino acids with a predicted size of 63 kD. The gene has also been mapped to human chromosome 6q14–21 (75). The cDNA predicts four N-linked glycosylation sites that likely account for the larger mass (69–71kD) of the mature form of the CD73 protein. Gutensohn et al. have shown by treatment with endoglycosidases and inhibitors of carbohydrate processing that human chorionic CD73 has four oligosaccharide side chains, all of which are N-linked (76). There is also evidence that CD73 is sialylated in some tissues (though probably not in lymphocytes), and there appears to be species- and tissue-specific variation in glycoslyation patterns (77). The 26 amino terminal residues specify a signal peptide, followed by the amino terminal sequence of the purified protein. The cDNA encodes a hydrophobic amino acid sequence at the carboxyl terminus that serves as a signal for the attachment of a GPI anchor.

CD73 has been remarkably conserved throughout evolution. The human cDNA predicts a protein that is 86%, 82%, 85%, and 64% identical to those encoded by the mouse (47), rat (64), bovine (65), and electic ray (66) cDNAs, respectively. In fact, CD73 contains motifs that are conserved in some bacterial hydrolases as well as consensus sequences for nucleotide-binding sites (4). Site-directed mutagenesis experiments revealed that conversion of any of three conserved histidines at amino acids 92, 194, or 217 to alanine resulted in loss of 5'-NT enzyme activity for human CD73 (78).

CD73 regulation

As discussed above, CD73 is developmentally regulated in human B and T lymphocytes. In an attempt to understand why the level of CD73 expression changes during T-lymphocyte maturation, we made transgenic mice which overexpress CD73 in thymocytes (79). Even though the enzyme was highly expressed on cortical thymocytes which are normally CD73$^-$, and the overall level of enzyme activity in the thymus was 100-fold higher than in normal mice, there were no apparent abnormalities in either T-cell development or mature T-cell function. Thus, it does not seem to be important to protect thymocytes from the products of 5'-NT enzyme activity early in their development. The high level of ADA in the thymus can apparently handle whatever adenosine is produced by 5'-NT, even if 5'-NT is highly overexpressed.

In addition to developmental control, there is also tissue- and cell-type-specific regulation of CD73 expression. While CD73 is expressed in many tissues, including the placenta, liver, and kidney, only certain cell types within each tissue express the protein (3). By immunohistochemistry, CD73 is selectively expressed on endothelial cells of the kidney, spleen and liver, on the basal layer of non-keratinizing squamous epithelium and transitional cell mucosa, and on the brush border of jejunal and ileal enterocytes. In some tissues, ADA also undergoes cell-type-specific regulation and is expressed primarily in cells which are 5'-NT$^-$. One example is the murine decidua. The primary decidua is 5'-NT^{hi} and ADA^{lo}; the adjacent secondary decidua is 5'-NT^{lo} and ADA^{hi} (80). Another example is the retina where 5'-NT is primarily in the epithelial and outer segment layers while ADA is highest in the cell bodies of the inner nuclear and ganglion cell layers (81). Finally, in the thymus, the cortex is ADA^{hi} 5'-NT^{lo} and the medulla is ADA^{lo} 5'-NT^{hi} (46, 82)*. We speculate that 5'-NT and ADA may be coordinately regulated in these tissues in an inverse fashion in order to control adenosine levels. Consistent with this hypothesis, Spychala et al. (83) have shown that PMA-induced HL-60 cell differentiation is accompanied by an increase in 5'-NT

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activity and a decrease in ADA activity, leading to an increase in intracellular adenosine levels. They propose that the 5'-NT/ADA enzymatic profile of a cell characterizes it as "adenosine-utilizing" (5'-NT^{lo} ADA^{hi}) or "adenosine-producing" (5'-NT^{hi} ADA^{lo}).

These observations suggest that understanding how expression of CD73 is regulated may lend insight into the molecular mechanisms responsible for normal lymphocyte development and the control of adenosine receptor engagement (see below). Comparisons between 5'-NT enzyme activity and CD73 mRNA levels in murine tissues (47) suggested that CD73 protein expression is controlled principally by steady-state mRNA levels, rather than by translational or post-translational mechanisms. In order to characterize the transcriptional regulation of human CD73, we cloned and characterized the promoter and 5'-flanking region of the gene (84). A cluster of potential transcription start sites was identified by RNase protection assays and 5'-RACE (rapid amplification of cDNA ends) cloning. The genomic region upstream from the CD73 start codon is GC-rich, and there is no TATAA box. There are five consensus Sp-1-binding sites, one cAMP-responsive element, two AP-2-binding sites, and one CCAAT box in the first 500 base pairs upstream from the transcriptional start site. The region between −1902 and −950 (with +1 as the site of transcription initiation) is also rich in binding sites for transcription factors, including three C/EBP sites, three GATA boxes, two MyoD sites, and inverted IgH heptamers and NFκB-binding sites. Nuclear run-on assays revealed high levels of transcription initiation in human placenta (strongly CD73$^+$), and undetectable levels in cell lines that expressed no (Jurkat) or low levels (WI-L2) of CD73. This suggested that CD73 levels are regulated, at least in part, by transcriptional initiation. However, our inability to detect differences in the levels of nascent transcripts in Jurkat and WI-L2 leaves open the possibility that additional mechanisms (RNA stability, RNA transport, transcriptional attenuation, or post-translational control) may govern CD73 protein levels in lymphocytes. In fact, transient transfection of luciferase reporter constructs demonstrated that a 155 base pair genomic DNA segment that included the transcription initiation site functions as a core promoter in both CD73$^+$ (MG and WI-L2) and CD73$^-$ (Raji and Jurkat) lymphoid cell lines. Additional genomic DNA sequences extending as far as 1.9 kb did not confer cell-type-specific expression to this core promoter in luciferase assays. While it is possible that elements regulating transcription are present outside this 1.9 kb region, or are present but do not function outside of the normal chromatin structure, it is equally likely that mechanisms beyond transcriptional initiation control the intricate regulation of CD73 levels in human lymphocytes.

One such mechanism has been proposed by Peola et al. (85). They found that treating lymphoid cell lines, peripheral blood T cells, or thymocytes with anti-CD38 mAb led to a dramatic increase in enzymatically active cell-surface CD73. Upregulation occurred in the presence of the protein synthesis inhibitor cycloheximide and was seen as quickly as 20 min after mAb treatment, declining to baseline by 12 h. These provocative data, which suggest that CD73 expression may in some circumstances be controlled by post-translational mechanisms, await confirmation by other groups.

CD73 functions

CD73 functions can be divided into two types: those which require 5'-NT enzyme activity and those which do not. In the first category, putative functions for which there is *in vitro* evidence include the synthesis of nucleosides for purine salvage and the production of adenosine to engage adenosine receptors. Postulated functions for CD73 which are independent of enzyme activity are the transduction of signals across the plasma membrane and cell adhesion (Fig. 1).

Purine salvage

Nucleoside monophosphates cannot cross the cell membrane, while nucleosides can enter the cell via facilitated diffusion. Thus, 5'-NT can convert nucleoside monophosphates into a form in which they can be utilized to meet the metabolic needs of cells (86). In fact, the enzymatic activity of ecto-5'-NT on mitogen-stimulated human peripheral blood T cells or EBV-transformed B-lymphoblastoid cell lines is sufficient to supply the total purine requirements of these rapidly dividing cells. IMP, inosine, and hypoxanthine (see Fig. 1) at 30 μM were all equally effective in supporting the proliferation of cells in which *de novo* purine synthesis was blocked by aminopterin (87). Inosine and hypoxanthine can enter cells directly, while IMP must first be dephosphorylated to inosine by 5'-NT. These studies addressed the magnitude of ecto-5'-NT enzyme activity on lymphocytes, but did not deal with the equally important issue of substrate availability.

Generally, nucleotides, including 5'-NT substrates, are found primarily inside cells. Since the catalytic site of ecto-5'-NT is on the outside of the plasma membrane (88), this raised the question of whether nucleotide substrates would normally be available to the enzyme. In 1985, we speculated that extracellular nucleotides might be available in tissues such as the thymus, bone marrow, and spleen where high rates of

apoptosis occur. Indeed, there are now several documented circumstances where 5'-NT has access to extracellular nucleotides (see below). These include the murine thymus as revealed by our recent analysis of nucleoside levels in thymuses of transgenic mice overexpressing human CD73 under the control of the *lck* promoter (79). Although adenosine levels were not changed in these mice, thymic inosine concentrations were increased more than 2-fold. The extremely high activity of ADA in the thymus converted adenosine generated by 5'-NT into inosine (see *Fig.* 1). This demonstrated for the first time the availability in the thymus of the 5'-NT substrate AMP.

CD73 produces adenosine to interact with adenosine receptors

Adenosine receptors are seven transmembrane-spanning G-protein-coupled receptors. They are widely expressed on a variety of tissues and cell types and mediate many important physiological responses, including cardiac rate and contractility, neurotransmission, renal function, smooth muscle vasodilation, platelet aggregation, superoxide anion generation, lipolysis, and mast cell activation (89–92). There are four known adenosine receptors which have been cloned and sequenced: A1, A2a, A2b, and A3. They are classified according to their affinities for adenosine and a variety of adenosine analogues and by their coupling to adenylate cyclase (93). All except the A1 receptor are expressed in murine thymus throughout development as revealed by reverse transcription-polymerase chain reaction, northern blotting, and/or immunohistochemistry or western blotting (94–96). Both the A2a and A2b receptors are positively coupled to adenylate cyclase and can mediate increases in cAMP. The A2a receptor has a much higher affinity for adenosine than the A2b receptor (97) and is more likely to be engaged at normal physiological concentrations of adenosine. The A3 receptor is negatively coupled to adenylate cyclase and has a low affinity for adenosine (93, 98). Both the A2b and A3 receptors can also signal through phospholipase C and/or ion fluxes (93).

Outside the immune system, there are several circumstances in which CD73 has been demonstrated to regulate the concentrations of extracellular adenosine which can engage adenosine receptors. For example, CD73 plays a crucial role in the T84 intestinal epithelial cell model of secretory diarrhea (99). In this system, adenosine is produced from neutrophil-derived AMP by CD73 on the surface of T84 cells. Adenosine then interacts with adenosine A2b receptors on these same cells (100) to induce electrogenic chloride secretion. CD73 on intestinal epithelial cells may, thus, play an important role *in vivo* in regulating the production of adenosine from AMP (or ATP) released from neutrophils at sites of intestinal inflammation. CD73 is also responsible for the production of adenosine which is important for the anti-inflammatory action of methotrexate in the *in vivo* murine air pouch model of inflammation*. Similarly, myocardial CD73 plays an important role in generating adenosine responsible for the phenomenon of ischemic preconditioning mediated by A1 receptors in studies of cardiac ischemia in dogs (101, 102).

Finally, ADA and CD73 appear to work together to regulate extracellular adenosine levels in the developing murine gestation site (80). The concentrations of adenosine in the developing murine embryo-decidual unit are proportional to the 5'-NT: ADA mRNA ratio, and it is tempting to speculate that these two genes may be co-ordinately regulated in this tissue. Adenosine levels rise dramatically on day 5 of gestation at the time 5'-NT expression is induced in the primary decidua and then fall as 5'-NT activity declines and ADA is induced in the adjacent secondary decidua (days 7–11). In this case, the extracellular AMP is probably generated through apoptosis, as there is a great deal of cell death when the decidua regresses and the placenta assumes the nourishment of the developing embryo. The A2b receptor is induced in the gestation site with kinetics which are virtually identical to those of 5'-NT, suggesting that 5'-NT and ADA regulate the amount of adenosine which can interact with this receptor†.

The cell-type-specific expression of 5'-NT and ADA in the murine gestation site is reminiscent of the thymus where 5'-NT expression is high and ADA expression is low in the medulla, and the converse is true in the cortex (46, 82)‡. *In vitro* studies in the mouse have shown that engagement of adenosine receptors on thymocyte suspensions induces apoptosis (79, 103, 104). Furthermore, cAMP-elevating agents block thymocyte differentiation in murine fetal thymic organ culture (105), suggesting that the regulation of intrathymic adenosine levels may be important for normal thymic development. We demonstrated, using thymocytes from our transgenic mice which overexpress human CD73, that adenosine produced by 5'-NT can engage adenosine receptors. As shown in *Fig.* 2, incubation of transgenic, but not control thymocytes, with AMP led to increases in intracellular cAMP. Furthermore, the dose response for AMP is very similar to that for adenosine, suggesting that

* Morabito L, et al. Methotrexate and sulfasalazine promote adenosine release by a mechanism that requires ecto-5'-nucleotidase-mediated conversion of adenine nucleotides. J Clin Invest (In press).

† Blackburn MR, Wubah JA, Thompson LF, Knudsen TB. Developmental expression of adenosine A2b receptors during implantation and placentation in the mouse. (Manuscript in preparation).

‡ Yamashita Y, et al. Expression of ecto-5'-nucleotidase (CD73) on murine leukocytes and stromal cells. (Manuscript in preparation).

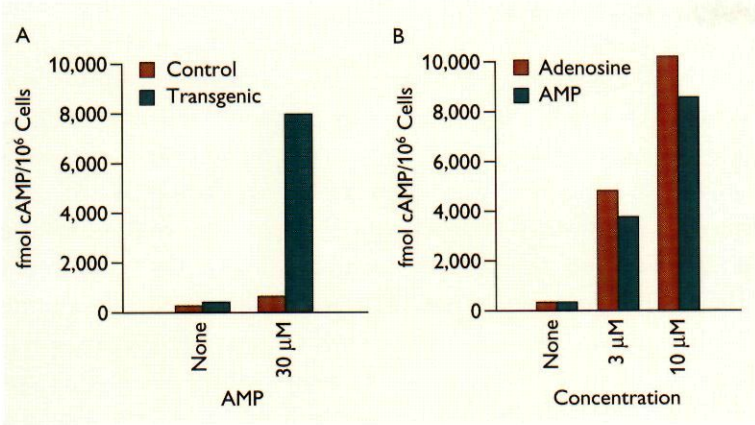

Fig. 2. 5'-NT can produce adenosine which engages adenosine receptors. Thymocyte suspensions were treated with the indicated concentrations of adenosine or AMP for 20 min. cAMP levels were measured with a commercial ELISA kit. **A.** Thymocytes from transgenic mice overexpressing 5'-NT and control littermates were compared for intracellular cAMP levels after exposure to 30 μM AMP. **B.** Thymocytes from transgenic mice overexpressing 5'-NT were incubated with the indicated concentrations of adenosine or AMP, and the changes in cAMP levels were compared.

the coupling of the enzymatic activity of 5'-NT to adenosine receptor signaling is quite efficient. It will be important to determine whether adenosine receptor signaling by 5'-NT-generated adenosine plays a role in regulating the high rate of apoptosis which normally occurs during thymic development.

It is possible that aberrant adenosine receptor signaling is involved in the pathogenesis of SCID caused by mutations in the ADA gene (79, 106). Both adenosine and deoxyadenosine are elevated in ADA-deficient patients (16) and adenosine levels are elevated in ADA knockout mice (107–109) or normal mice treated with the ADA inhibitor 2-deoxycoformycin (79) to the point where one would expect adenosine receptors to be engaged. We are currently evaluating the role of adenosine receptor signaling in the pathogenesis of ADA deficiency using murine fetal thymic organ culture. If elevated thymic adenosine levels are important in ADA deficiency, it will be important to understand the relative roles of ecto- versus cytoplasmic nucleotidases in their production. It is possible that the consequences of ADA deficiency could be ameliorated by a decrease of 5'-NT activity in the thymus.

Huang et al. recently reported that treatment of splenocytes with the A2a receptor agonist CGS21680 leads to an inhibition of antigen-induced T-cell proliferation (110). These authors argued that adenosine receptor-mediated peripheral T-cell depletion might be important in the pathogenesis of SCID caused by ADA deficiency. However, we feel this is unlikely. The extreme thymic atrophy in ADA-deficient patients (16) suggests that a block in early thymocyte maturation is primarily responsible for their T lymphopenia.

CD73 is a co-receptor for CD3/TCR

Cross-linking CD73 by either polyclonal or monoclonal antibodies in the presence of PMA caused T cells to proliferate, express the IL-2R, and secrete IL-2 (111). The proliferation was as intense as with PHA stimulation. Both intact IgG and $F(ab')_2$ fragments were stimulatory, and proliferation occurred with even monocyte-depleted (<0.3%) T-cell preparations. These results suggested that CD73 could transmit transmembrane signals.

Our original observations were extended to a system in which anti-CD73 mAbs were co-immobilized with the anti-CD3 mAb OKT3 (112). Though immobilized anti-CD73 alone did not cause T cells to proliferate, T-cell proliferation was intense when anti-CD73 was co-immobilized with submitogenic concentrations of anti-CD3 or when suboptimal concentrations of soluble anti-CD2 mAbs were added to the system. A comparison with the well-characterized co-stimulatory molecule CD28 (113) revealed that T-cell proliferative responses were equivalent or greater when T cells were co-stimulated with immobilized anti-CD3 plus anti-CD73 mAbs than with anti-CD3 plus anti-CD28. Subsequent studies revealed that CD73 was primarily expressed on naive $CD45RA^{hi}$ $CD45RO^{lo}CD8^{+}$ T cells (28). These cells have an intrinsic low responsiveness to activation through immobilized anti-CD3 which can be markedly enhanced by co-immobilization of anti-CD73. $CD8^{+}$ memory cells ($CD45RA^{lo}CD45RO^{hi}$), on the other hand, responded well to immobilized anti-CD3 alone and this response was not increased by the addition of immobilized anti-CD73. These results suggested that one function of CD73 may be to lower the threshold for the activation of naive T cells when they first encounter antigen.

Thus, CD73 stimulates T cells by amplifying activation signals delivered through CD3 or CD2 and is part of a family of stimulatory molecules that transduce such activating signals in T lymphocytes. These molecules include CD28 (113), Thy-1 (114), and T-cell-activating protein (TAP) (115). CD73 substrates could not substitute for anti-CD73 in these experiments, suggesting that there might be a non-substrate ligand for

CD73. Anti-CD73 mAbs may mimic the actions of this natural ligand, as has been shown for some T-cell co-stimulatory molecules and their ligands such as CD28 and B7-1/B7-2 (116).

Many other T-cell co-activating molecules, including Thy-1 (114), TAP (115), Ly-6 (117), Qa-2 (118), RT-6 (119), CD48 (120), CD55 (121), and CD59 (122), are also GPI-anchored. This has led many investigators to propose that there may be a direct role for the GPI anchor in transmembrane signaling (67). We established a system to evaluate the role of the GPI anchor in the transmission of signals via CD73. The CD73$^-$ human T-cell line Jurkat was transfected with cDNAs for both conventional GPI-anchored CD73 and a transmembrane form of the molecule engineered by fusing the extracellular portion of CD73 to the transmembrane portion of human tissue factor (123). The activation of Jurkat cells after incubation with CD73 mAb plus PMA for 24 h was measured by the secretion of IL-2. We found that the GPI anchor facilitated, but was not necessary for, the activation of Jurkat cells via CD73. The activation of CD73-transfected Jurkat cells was not dependent upon 5'-NT enzyme activity (124). Clones of transfectants expressing enzyme-inactive CD73 with His→Ala substitutions at amino acids 92 or 194 could be activated to secrete as much IL-2 as clones expressing wild-type CD73. Using a panel of Jurkat mutants, we found that the TCR, the tyrosine kinase lck, and the tyrosine phosphatase CD45 are all required for Jurkats to signal through CD73 (123). These data demonstrate that the signals delivered to the T cell through CD73 require an intact CD3-signaling pathway, and suggest that the extracellular domain of CD73 may interact with the CD3/TCR complex either directly or indirectly.

Cell adhesion

There is some evidence that CD73 might function as an adhesion molecule. Several glycoproteins important in cell-cell and cell-matrix contact, including N-CAM and cytoactin, have been noted to react with the mAb HNK-1. The epitope recognized by the HNK-1 mAb has been identified as glucuronic acid 3-sulfate. Ecto-5'-NT from electric ray and cat brain are also reactive with HNK-1, suggesting that CD73 from these sources carries an epitope implicated in cell adhesion (125).

CD73 isolated from chicken gizzard bound to fibronectin and laminin, and this binding was blocked by anti-CD73 antibodies (126). Laminin and fibronectin also altered ecto-5'-NT enzyme activity from chicken gizzard smooth muscle (127). Chicken gizzard CD73 reconstituted into proteoliposomes was shown by Stochaj et al. (128) to specifically bind fibronectin. However, the ability of laminin or fibronectin to serve as natural ligands for CD73 on mammalian lymphocytes is unproven.

CD73 may make a small contribution to the binding of lymphocytes to endothelial cells (44). Airas et al. (44) generated a CD73-specific mAb which inhibited chromium-labeled lymphocyte binding to human umbilical vein endothelial cells by approximately 25%. Thirty-two percent of fluorescently labeled human lymphocytes bound to CD73-transfected COS cells, compared to 25% for mock-transfected cells. This weak recognition was only partially blocked by an anti-CD73 mAb (71). This same group also argued that CD73 has a role in the adhesion of B cells to FDC (50). Anti-CD73 mAb modestly reduced aggregation of dendritic cell preparations from tonsillar germinal centers (5–15% dendritic cells, 80–90% B cells) in an adhesion assay scored by microscopic inspection of fixed cells. These data are interesting, but the exact cells aggregating in this FDC-B-cell preparation were not identified. A more robust experimental system will be required to demonstrate conclusively that this molecule plays an important role in modulating B-cell-FDC interactions.

Areas for future investigation

We have summarized much of what is known about the role of CD73 in the immune system. Human CD73 is expressed on subsets of T and B lymphocytes, on germinal center FDC, and on reticular fibroblasts and epithelial cells of the thymic medulla. It is an ecto-enzyme that generates nucleosides both for purine salvage and for adenosine receptor-mediated signaling. Independent of enzyme activity, CD73 transduces signals across the T-cell membrane, and may mediate cell adhesion. Many important questions about the role of CD73 in the immune system, though, remain unanswered.

CD73 expression on lymphocytes is intricately regulated. What upregulates CD73 expression when T cells leave the thymus? Similarly for B cells, is 5'-NT enzyme activity needed for differentiation or is its presence merely a marker of certain developmental stages? There have been no tissue-specific promoter or enhancer elements identified for the 5'-NT gene. It is also intriguing to consider that there may be co-ordinate regulation with ADA that controls adenosine levels in a given tissue. The mechanisms controlling tissue-specific expression of CD73 are of great interest given the ability of adenosine to modulate a wide variety of physiological responses through adenosine receptor engagement.

All CD73$^+$ T cells express the powerful T-cell co-stimulatory molecule CD28. A better understanding of the function of CD73 on mature T cells may explain the intriguing observation that some T cells express the enzyme while others do not. Additionally, data suggest that there is an unidentified natural

ligand for CD73 which transmits activation signals that synergize with signals generated through the TCR. Attempts to find a ligand should now be made in the mouse where secondary lymphoid tissue and bone marrow (which may contain ligand-bearing cells) are readily obtainable and where an anti-murine CD73 is newly available.

Understanding why CD73 is GPI-anchored may lend insight into the biology of this mode of membrane attachment. There might be circumstances where it would be advantageous to have rapid loss of a molecule from the cell surface through cleavage by a PI-PLC. CD73 is, in fact, rapidly lost from human lymphocytes when they are incubated with immobilized anti-CD73 mAbs. However, this phenomenon occurs equally well with Jurkat cells transfected with GPI-anchored and transmembrane forms of CD73, suggesting that it is mediated by a protease rather than a phospholipase (L.F. Thompson, unpublished observations). The GPI-anchored protein CD16 has also been found to be removed from the cell surface by proteolytic cleavage (129). Alternatively, the GPI anchor could be involved in signal transduction. However, unlike CD55 (130), Ly-6A (131), and Qa-2 (132) where the GPI-anchor appears to be essential, a transmembrane form of CD73 can also transmit signals across the membrane (123). A third postulated function for the GPI anchor is to provide increased lateral mobility. The substitution of a conventional transmembrane anchor for the normal GPI anchor reduced the lateral mobility of Thy-1 by a factor of 2 (133). Adenosine produced by GPI-anchored 5'-NT is efficiently coupled to adenosine receptor signaling, resulting in increased intracellular levels of cAMP (Fig. 2). It would be interesting to repeat this experiment with a transmembrane form of CD73 to see if it were equally efficient.

Finally, there are several interesting issues regarding CD73 with respect to hematologic malignancies. First, do patients with $CD73^+$ CLL have a different prognosis than those with $CD73^-$ cells? Second, is CD73 expression needed for the maintenance of the multidrug-resistant phenotype in cancer patients as was shown for doxorubicin-resistant cell lines (58, 59)? Third, is the level of CD73 expression on leukemic cells consistent with their state of differentiation, or is the gene aberrantly regulated? In any case, the clear $CD73^+$ or $CD73^-$ nature of certain lymphoid or myeloid malignancies might be exploited in the design of therapeutic agents.

Future experiments will take advantage of transgenic technology. Gene-targeted mice will allow analysis of the function of CD73 in both the immune system and the entire organism. Tissue-specific targeting may be even more revealing by allowing a dissection of CD73 function within the immune system. Additionally, mice expressing enzymatically inactive CD73 will allow an assessment of the enzymatic versus signaling or adhesive functions of this protein. Answers to all these questions will yield insight into the function of CD73 in the immune system, as well as in the variety of biological processes in which this fascinating molecule participates.

References

1. Burger RM, Lowenstein JM. 5'-nucleotidase from smooth muscle of small intestine and from brain. Inhibition by nucleotides. Biochemistry 1975;**14**:2362–2366.
2. Naito Y, Lowenstein JM. 5'-nucleotidase from rat heart. Biochemistry 1981;**20**:5188–5194.
3. Thompson LF, et al. Production and characterization of monoclonal antibodies to the glycosyl phosphatidylinositol-anchored lymphocyte differentiation antigen ecto-5'-nucleotidase (CD73). Tissue Antigens 1990;**35**:9–19.
4. Zimmermann H. 5'-nucleotidase: Molecular structure and functional aspects. Biochem J 1992;**285**:345–365.
5. Silber R, Conklyn M, Grusky G, Zucker-Franklin D. Human lymphocytes: 5'-nucleotidase-positive and -negative subpopulations. J Clin Invest 1975;**56**:1324–1327.
6. Kramers MTC, Catovsky D, Cherchi M, Galton DAG. 5' nucleotidase in cord blood lymphocytes. Leuk Res 1977;**1**:279–281.
7. Salmon SE, Seligmann M. B-cell neoplasia in man. Lancet 1974;**2**:1230–1233.
8. DePierre JW, Karnovsky ML. Ecto-enzymes of the guinea pig polymorphonuclear leukocyte. II. Properties and suitability as markers for the plasma membrane. J Biol Chem 1974;**249**:7121–7129.
9. Johnson SM, Asherson GL, Watts RWE, North ME, Allsop J, Webster ADB. Lymphocyte purine 5'-nucleotidase deficiency in primary hypogammaglobulinemia. Lancet 1977;**1**:168–170.
10. Edwards NL, Magilavy DB, Cassidy JT, Fox IH. Lymphocyte ecto-5'-nucleotidase deficiency in agammaglobulinemia. Science 1978;**201**:628–630.
11. Cunningham-Rundles C. Clinical and immunologic analyses of 103 patients with common variable immunodeficiency. J Clin Immunol 1989;**9**:22–33.
12. Campana D, Farrant J, Inamdar N, Webster ADB, Janossy G. Phenotypic features and proliferative activity of B cell progenitors in X-linked agammaglobulinemia. J Immunol 1990;**145**:1675–1680.
13. Giblett ER, Anderson JE, Cohen F, Pollara B, Meuwissen HJ. Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet 1972;**2**:1067–1069.
14. Giblett ER, Ammann AJ, Sandman R, Wara DW, Diamond LK. Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. Lancet 1975;**1**:1010–1013.
15. Edwards NL, Cassidy JT, Fox IH. Lymphocyte 5'-nucleotidase deficiency in hypogammaglobulinemia: Clinical characteristics. Clin Immunol Immunopathol 1980;**17**: 76–88.

16. Hershfield MS, Mitchell BS. Immunodeficiency diseases caused by adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill, Inc. 1995. p. 1725–1768.
17. Thompson LF, et al. Ecto-5'-nucleotidase activity in T and B lymphocytes from normal subjects and patients with congenital X-linked agammaglobulinemia. J Immunol 1979;**123**:2475–2478.
18. Rowe M, De Gast CG, Platts-Mills TAE, Asherson GL, Webster ADB, Johnson SM. 5'-nucleotidase of B and T lymphocytes isolated from human peripheral blood. Clin Exp Immunol 1979;**36**:97–101.
19. Thompson LF, Ruedi JM, O'Connor RD, Bastian JF. Ecto-5'-nucleotidase expression during human B cell development. An explanation for the heterogeneity in B lymphocyte ecto-5'-nucleotidase activity in patients with hypogammaglobulinemia. J Immunol 1986;**137**:2496–2500.
20. Rowe M, DeGast GC, Platts-Mills TAE, Asherson GL, Webster ADB, Johnson SM. Lymphocyte 5'-nucleotidase in primary hypogammaglobulinemia and cord blood. Clin Exp Immunol 1980;**39**:337–343.
21. Bastian JF, Ruedi JM, MacPherson GA, Golembesky HE, O'Connor RD, Thompson LF. Lymphocyte ecto-5'-nucleotidase activity in infancy: increasing activity in peripheral blood B cells precedes their ability to synthesize IgG in vitro. J Immunol 1984;**132**:1767–1772.
22. Edwards NL, Gelfand EW, Burk L, Dosch H-M, Fox IH. Distribution of 5'-nucleotidase in human lymphoid tissues. Proc Natl Acad Sci USA 1979;**76**:3474–3476.
23. Thompson LF, Saxon A, O'Connor RD, Fox RI. Ecto-5'-nucleotidase activity in human T cell subsets. Decreased numbers of ecto-5'-nucleotidase positive cells from both OKT4+ and OKT8+ cells in patients with hypogammaglobulinemia. J Clin Invest 1983;**71**:892–899.
24. De La Concha EG, Oldham G, Webster ADB, Asherson GL, Platts-Mills TAE. Quantitative measurements of T- and B-cell function in "variable" primary hypogammaglobulinemia: evidence for a consistent B-cell defect. Clin Exp Immunol 1977;**27**:208–215.
25. Tsukada S, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell 1993;**72**:279–290.
26. Recker DP, Edwards NL, Fox IH. Histochemical evaluation of lymphocytes in hypogammaglobulinemia. Decreased number of 5'-nucleotidase-positive cells. J Lab Clin Med 1980;**95**:175–179.
27. Matamoros N, Horwitz DA, Newton C, Asherson GL, Webster ADB. Histochemical studies for 5'-nucleotidase and alpha-naphthyl (non-specific) esterase in lymphocytes from patients with primary immunoglobulin deficiencies. Clin Exp Immunol 1979;**36**:102–106.
28. Dianzani U, et al. Co-stimulatory signal delivered by CD73 molecule to human $CD45RA^{hi}CD45RO^{lo}$ (naive) CD8+ T lymphocytes. J Immunol 1993;**151**:3961–3970.
29. Thompson LF, Ruedi JM, Glass A, Lucas AH. Production of mAb to ecto-5'-nucleotidase: a glycosyl phosphatidylinositol-anchored differentiation antigen expressed on human T- and B-lymphocytes. In: Knapp W, et al. eds. Leucocyte Typing IV: white cell differentiation antigens. New York: Oxford University Press; 1989. p. 104–106.
30. Bastian JF, Williams RA, Ornelas W, Tani P, Thompson LF. Maternal isoimmunization resulting in an infant with combined immunodeficiency and fatal graft versus host disease. Lancet 1984;**1**:1435–1437.
31. Boss GR, Thompson LF, O'Connor RD, Ziering RW, Seegmiller JE. Ecto-5'-nucleotidase deficiency: Association with adenosine deaminase deficiency and non-association with deoxyadenosine toxicity. Clin Immunol Immunopathol 1981;**19**:1–7.
32. Thompson LF, O'Connor RD, Bastian JF. Phenotype and function of engrafted maternal T cells in patients with severe combined immunodeficiency. J Immunol 1984;**133**:2513–2517.
33. Cohen A, Mansour A, Dosch H-M, Gelfand EW. Association of a lymphocyte purine enzyme deficiency (5'-nucleotidase) with combined immunodeficiency. Clin Immunol Immunopathol 1980;**15**: 245–250.
34. Gelfand EW, McCurdy D, Rao CP, Cohen A. Absence of lymphocyte ecto-5'-nucleotidase in infants with reticuloendotheliosis and eosinophilia (Omenn's Syndrome). Blood 1984;**63**:1475–1480.
35. Salazar-Gonzalez JF, Moody DJ, Giorgi JV, Martinez-Maza O, Mitsuyasu RT, Fahey JL. Reduced ecto-5'-nucleotidase activity and enhanced OKT10 and HLA-DR expression on CD8 (T suppressor/cytotoxic) lymphocytes in the acquired immune deficiency syndrome: Evidence of CD8 cell immaturity. J Immunol 1985;**135**:1778–1785.
36. Murray JL, Reuben JM, Munn CG, Mansell PW, Newell GR, Hersh EM. Decreased 5' nucleotidase activity in lymphocytes from asymptomatic sexually active homosexual men and patients with the acquired immune deficiency syndrome. Blood 1984;**64**:1016–1021.
37. Anderson SJ, Perlmutter RM. A signaling pathway governing early thymocyte maturation. Immunol Today 1995;**16**:99–105.
38. Pfeffer K, Mak TW. Lymphocyte ontogeny and activation in gene targeted mutant mice. Annu Rev Immunol 1994;**12**:367–411.
39. Thompson LF, Ruedi JM, Low MG, Clement LT. Distribution of ecto-5'-nucleotidase on subsets of human T and B lymphocytes as detected by indirect immunofluorescence using goat antibodies. J Immunol 1987;**139**:4042–4048.
40. Kummer U, et al. Development and properties of a monoclonal antibody specific for human ecto-5'-nucleotidase. Immunobiology 1984;**166**:203–211.
41. Dorken B, Moller P, Pezzutto A, Schwartz-Albiez R, Moldenhauer G. B cell antigens: CD73. In: Knapp W, et al. eds. Leucocyte Typing IV: white cell differentiation antigens. New York: Oxford University Press; 1989. p. 102–104.
42. Fukunaga Y, et al. Increased density of ecto-5'-nucleotidase antigen on leukemic T cells from patients with cutaneous T cell lymphoma and adult T cell leukemia/lymphoma. Blood 1989;**74**:2486–2492.
43. Thompson LF, Laurent AB, Franklin MK, Gutensohn W, Resta R. CD73 workshop panel report. In: Schlossman SF, et al. eds. Leucocyte Typing V. New York City: Oxford University Press, Inc. 1995. p. 564–567.
44. Airas L, Salmi M, Jalkanen S. Lymphocyte-vascular adhesion protein-2 is a novel 70-kDa molecule involved in lymphocyte adhesion to vascular endothelium. J Immunol 1993;**151**:4228–4238.
45. Gandhi R, LeHir M, Kaissling B. Immunolocalization of ecto-5'-nucleotidase in the kidney by a monoclonal antibody. Histochemistry 1990;**95**:165–174.
46. Ma DDF, et al. Distribution of terminal deoxynucleotidyl transferase and purine degradative and synthetic enzymes in subpopulations of human thymocytes. J Immunol 1982;**129**:1430–1435.
47. Resta R, et al. Murine ecto-5'-nucleotidase (CD73): cDNA cloning and tissue distribution. Gene 1993;**133**:171–177.
48. Oleinik BV, Tarasova AA, Bezvershenko IA. Activity of cell-detached 5'-nucleotidase in thymocytes of various densities. Tsitologiia 1983;**25**:106–110.

49. Dianzani U, Massaia M, Pileri A, Grossi CE, Clement LT. Differential expression of ecto-5'-nucleotidase activity by functionally and phenotypically distinct subpopulations of human Leu-2+/T8+ lymphocytes. J Immunol 1986;**137**:484–489.
50. Airas L, Jalkanen S. CD73 mediates adhesion of B cells to follicular dendritic cells. Blood 1996;**88**:1755–1764.
51. Barton RW, Goldschneider I. 5'-nucleotidase activity in subpopulations of rat lymphocytes. J Immunol 1978;**121**:2329–2334.
52. Thompson LF, Ruedi JM. Functional characterization of ecto-5'-nucleotidase positive and negative human T lymphocytes. J Immunol 1989;**142**:1518–1522.
53. Thompson LF, Ruedi JM. Synthesis of immunoglobulin G by pokeweed mitogen- or Epstein-Barr virus-stimulated human B cells in vitro is restricted to the ecto-5'-nucleotidase positive subset. J Clin Invest 1988;**82**:902–905.
54. Pieters R, et al. Expression of 5'-nucleotidase (CD73) related to other differentiation antigens in leukemias of B-cell lineage. Blood 1991;**78**:488–492.
55. Veerman AJP, Hogeman PHG, van Zantwijk CH, Bezemer PD. Prognostic value of 5'nucleotidase in acute lymphoblastic leukemia with the common-all phenotype. Leuk Res 1985;**9**:1227–1229.
56. Gutensohn W, Thiel E. Prognostic implication of ecto-5'-nucleotidase activity in acute lymphoblastic leukemia. Cancer 1990;**66**:1755–1758.
57. Pieters R, et al. Relation of 5'-nucleotidase and phosphatase activities with immunophenoptype, drug resistance and clinical prognosis in childhood leukemia. Leuk Res 1992;**16**:873–880.
58. Ujházy P, Klobusicka M, Babusikova O, Strausbauch P, Mihich E, Ehrke MJ. Ecto-5'-nucleotidase (CD73) in multidrug resistant cell lines generated by doxorubicin. Int J Cancer 1994;**59**:83–93.
59. Ujházy P, et al. Evidence for the involvement of ecto-5'-nucleotidase (CD73) in drug resistance. Int J Cancer 1996;**68**:493–500.
60. Shukla SD, Coleman R, Finean JB, Michell RH. Selective release of plasma-membrane enzymes from rat hepatocytes by a phosphatidylinositol-specific phospholipase C. Biochem J 1980;**187**:277–280.
61. Klemens MR, Sherman WR, Holmberg NJ, Ruedi JM, Low MG, Thompson LF. Characterization of soluble vs membrane-bound human placental 5'-nucleotidase. Biochem Biophys Res Commun 1990;**172**: 1371–1377.
62. Misumi Y, Ogata S, Ohkubo K, Hirose S, Ikehara Y. Primary structure of human placental 5'-nucleotidase and identification of the glycolipid anchor in the mature form. Eur J Biochem 1990;**191**:563–569.
63. Vogel M, Kowalewski H, Zimmermann H, Hooper NM, Turner AJ. Soluble low-Km 5'-nucleotidase from electric-ray (Torpedo marmorata) electric organ and bovine cerebral cortex is derived from the glycosyl-phosphatidylinositol-anchored ecto-enzyme by phospholipase C cleavage. Biochem J 1992;**284**:621–624.
64. Misumi Y, Ogata S, Hirose S, Ikehara Y. Primary structure of rat liver 5'-nucleotidase deduced from the cDNA. Presence of the COOH-terminal hydrophobic domain for possible post-translational modification by glycophospholipid. J Biol Chem 1990;**265**:2178–2183.
65. Suzuki K, et al. Purification and cDNA cloning of bovine liver 5'-nucleotidase, a GPI-anchored protein, and its expression in COS cells. J Biochem (Tokyo) 1993;**113**:607–613.
66. Volknandt W, Vogel M, Pevsner J, Misumi Y, Ikehara Y, Zimmermann H. 5'-Nucleotidase from the electric ray electric lobe. Primary structure and relation to mammalian and procaryotic enzymes. Eur J Biochem 1991;**202**:855–861.
67. Robinson PJ. Phosphatidylinositol membrane anchors and T-cell activation. Immunol Today 1991;**12**:35–41.
68. Brown D. The tyrosine kinase connection: how GPI-anchored proteins activate T cells. Curr Opin Immunol 1993;**5**:349–354.
69. Burger RM, Lowenstein JM. Preparation and properties of 5'-nucleotidase from smooth muscle of small intestine. J Biol Chem 1970;**245**:6274–6280.
70. Madrid-Marina V, Fox IH. Human placental cytoplasmic 5'-nucleotidase. J Biol Chem 1986;**261**:444–452.
71. Buschette-Brambrink S, Gutensohn W. Human placental ecto-5'-nucleotidase: isoforms and chemical crosslinking products of the membrane-bound and isolated enzyme. Biol Chem Hoppe Seyler 1989;**370**:67–74.
72. Thompson LF, Ruedi JM, Low MG. Purification of 5'-nucleotidase from human placenta after release from plasma membranes by phosphatidylinositol-specific phospholipase C. Biochem Biophys Res Commun 1987;**145**: 118–125.
73. Airas L, et al. CD73 is involved in lymphocyte binding to the endothelium: Characterization of lymphocyte vascular adhesion protein 2 identifies it as CD73. J Exp Med 1995;**182**:1603–1608.
74. Grondal EJM, Zimmermann H. Purification, characterization and cellular localization of 5'-nucleotidase from Torpedo electric organ. Biochem J 1987;**245**:805–810.
75. Boyle JM, Hey Y, Grzeschik K-H, Thompson L, Munro E, Fox M. Regional localization of human ecto-5'-nucleotidase to chromosome 6q14-q21. Hum Genet 1989;**83**:179–180.
76. Burgemeister R, Danescu I, Gutensohn W. Glycosylation and processing of carbohydrate side chains of ecto-5'-nucleotidase in cultured human chorionic cells. Biol Chem Hoppe Seyler 1990;**371**:355–361.
77. Harb J, Meflah K, Duflos Y, Bernard S. Purification and properties of bovine liver plasma membrane 5'-nucleotidase. Eur J Biochem 1983;**137**:131–138.
78. Gutensohn W, Resta R, Misumi Y, Ikehara Y, Thompson LF. Structure-function relationships in the CD73 molecule. In: Schlossman SF, et al. eds. Leucocyte Typing V. New York City: Oxford University Press, Inc. 1995. p. 567–568.
79. Resta R, et al. Insights into thymic purine metabolism and adenosine deaminase deficiency revealed by transgenic mice over expressing ecto-5'-nucleotidase (CD73). J Clin Invest 1997;**99**:676–683.
80. Blackburn MR, Gao X, Airhart MJ, Skalko RG, Thompson LF, Knudsen TB. Adenosine levels in the postimplantation mouse uterus: quantitation by HPLC-fluorometric detection and spatiotemporal regulation by 5'-nucleotidase and adenosine deaminase. Dev Dyn 1992;**194**:155–168.
81. Blazynski C, Perez MR. Adenosine in vertebrate retina: Localization, receptor characterization, and function. Cell Mol Neurobiol 1991;**11**:463–484.
82. Chechik BE, Schrader WP, Minowada J. An immunomorphologic study of adenosine deaminase distribution in human thymus tissue, normal lymphocytes, and hematopoietic cell lines. J Immunol 1981;**126**:1003–1007.
83. Spychala J, Mitchell BS, Barankiewicz J. Adenosine metabolism during phorbol myristate acetate-mediated induction of HL-60 cell difference – Changes in expression pattern of adenosine kinase, adenosine deaminase, and 5'-nucleotidase. J Immunol 1997;**158**:4947–4952.
84. Hansen KR, Resta R, Webb CF, Thompson LF. Isolation and characterization of the promoter of the human 5'-nucleotidase (CD73)-encoding gene. Gene 1995;**167**:307–312.

85. Peola S, Borrione P, Matera L, Malavasi F, Pileri A, Massaia M. Selective induction of CD73 expression in human lymphocytes by CD38 ligation – A novel pathway linking signal transducers with ecto-enzyme activities.
J Immunol 1996;**157**:4354–4362.
86. Fleit H, Conklyn M, Stebbins RD, Silber R. Action of 5'-nucleotidase in the uptake of adenosine from AMP by human lymphocytes.
J Biol Chem 1975;**23**:8889–8892.
87. Thompson LF. Ecto-5'-nucleotidase can provide the total purine requirements of mitogen-stimulated human T cells and rapidly dividing human B lymphoblastoid cells.
J Immunol 1985;**134**:3794–3797.
88. DePierre JW, Karnovsky ML. Ecto-enzymes of the guinea pig polymorphonuclear leukocyte. I. Evidence for an ecto-adenosine monophosphatase, adenosine triphosphatase, and -p-nitrophenyl phosphates.
J Biol Chem 1974;**249**:7111–7120.
89. Olsson RA, Pearson D. Cardiovascular purinoceptors.
Physiol Rev 1990;**70**:761–845.
90. Ramkumar V, Pierson G, Stiles G. Adenosine receptors: clinical implications and biochemical mechanisms.
Prog Drug Res 1988;**32**:195–247.
91. Cronstein BN, Rosenstein ED, Kramer SB, Weissmann G, Hirschhorn R. Adenosine: A physiologic modulator of superoxide anion generation by human neutrophils. Adenosine acts via an A2 receptor on human neutrophils.
J Immunol 1985;**135**:1366–1371.
92. Ramkumar V, Stiles GL, Beaven MA, Ali H. The A_3 adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells.
J Biol Chem 1993;**268**:16887–16890.
93. Palmer TM, Stiles GL. Adenosine receptors.
Neuropharmacology 1995;**34**:683–694.
94. Jiang H, Puffinbarger NK, Resta R, Thompson LF. Adenosine receptor expression in developing murine thymus.
FASEB J 1996;**10**:A1047(Abstract).
95. Puffinbarger NK, et al. Production and characterization of multiple antigenic peptide antibodies to the adenosine A-2b receptor.
Mol Pharmacol 1995;**47**:1126–1132.
96. Stehle JH, Rivkees SA, Lee JJ, Weaver DR, Deeds JD, Reppert SM. Molecular cloning and expression of the cDNA for a novel A2-adenosine receptor subtype.
Mol Endocrinol 1992;**6**:384–393.
97. Bruns RF, Lu GH, Pugsley TA. Characterization of the A2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes.
Mol Pharmacol 1986;**29**:331–346.
98. Zhou Q, Li C, Olah ME, Johnson RA, Stiles GL, Civelli O. Molecular cloning and characterization of an adenosine receptor: The A3 adenosine receptor.
Proc Natl Acad Sci USA 1992;**89**:7432–7436.
99. Madara JL, et al. 5'-AMP is the neutrophil derived paracrine factor that elicits chloride secretion from T84 intestinal epithelial cell monolayers.
J Clin Invest 1993;**91**:2320–2325.
100. Strohmeier GR, Reppert SM, Lencer WI, Madara JL. The A_{2b} adenosine receptor mediates cAMP responses to adenosine receptor agonists in human intestinal epithelia.
J Biol Chem 1995;**270**:2387–2394.
101. Kitakaze M, Masatsugu H, Takashima S, Sato H, Michitoshi I, Kamada T. Ishemic preconditioning increases adenosine release and 5'-nucleotidase activity during myocardial ischemia and reperfusion in dogs.
Circulation 1993;**87**:208–215.
102. Kitakaze M, et al. Infarct size-limiting effect of ischemic preconditioning is blunted by inhibition of 5'-nucleotidase activity and attenuation of adenosine release.
Circulation 1994;**89**:1237–1246.
103. McConkey DJ, Orrenius S, Jondal M. Agents that elevate cAMP stimulate DNA fragmentation in thymocytes.
J Immunol 1990;**145**:1227–1230.
104. Kizaki H, Suzuki K, Tadakuma T, Ishimura Y. Adenosine receptor-mediated accumulation of cyclic AMP-induced T lymphocyte death through internucleosomal DNA cleavage.
J Biol Chem 1990;**265**:5280–5284.
105. Lalli E, Sassone-Corsi P, Ceredig R. Block of T lymphocyte differentiation by activation of the cAMP-dependent signal transduction pathway.
EMBO J 1996;**15**:528–537.
106. Resta R, Thompson LF. SCID: the role of adenosine deaminase deficiency.
Immunol Today 1997;**18**:371–374.
107. Wakamiya M, et al. Disruption of the adenosine deaminase gene causes hepatocellular impairment and perinatal lethality in mice.
Proc Natl Acad Sci USA 1995;**92**:3673–3677.
108. Migchielsen AAJ, et al. Adenosine-deaminase-deficient mice die perinatally and exhibit liver-cell degeneration, atelectasis and small intestinal cell death.
Nat Genet 1995;**10**:279–287.
109. Blackburn MR, Datta SK, Wakamiya M, Vartabedian BS, Kellems RE. Metabolic and immunologic consequences of limited adenosine deaminase expression in mice.
J Biol Chem 1996;**271**:15203–15210.
110. Huang S, Apasov S, Koshiba M, Sitkovsky M. Role of A2a extracellular adenosine receptor-mediated signaling in adenosine-mediated inhibition of T-cell activation and expansion.
Blood 1997;**90**:1600–1610.
111. Thompson LF, Ruedi JM, Glass A, Low MG, Lucas AH. Antibodies to 5'-nucleotidase (CD73), a glycosyl-phosphatidylinositol-anchored protein, cause human peripheral blood T cells to proliferate.
J Immunol 1989;**143**:1815–1821.
112. Massaia M, et al. Human T cell activation: synergy between CD73 (ecto-5'-nucleotidase) and signals delivered through CD3 and CD2 molecules.
J Immunol 1990;**145**:1664–1674.
113. Hara T, Fu SM, Hansen JA. Human T cell activation. A new activation pathway used by a major T cell population via a disulfide-bonded dimer of a 44 kilodalton polypeptide (9.3 antigen).
J Exp Med 1985;**161**:1513–1524.
114. Conzelmann A, Spiazzi A, Hyman R, Bron C. Anchoring of membrane proteins via phosphatidylinositol is deficient in two classes of Thy-1 negative mutant lymphoma cells.
EMBO J 1986;**5**:3291–3296.
115. Yeh ETH, Reiser H, Benacerraf B, Rock KL. The expression, function, and ontogeny of a novel T cell-activating protein, TAP, in the thymus.
J Immunol 1986;**137**:1232–1238.
116. Thompson CB. Distinct roles for the costimulaotry ligands B7-1 and B7-2 in T helper cell differentiation?
Cell 1995;**81**:979–982.
117. Malek TR, Ortega G, Chan C, Kroczek RA, Shevach EM. Role of Ly-6 in lymphocyte activation. Induction of T cell activation by monoclonal anti-Ly-6 antibodies.
J Exp Med 1986;**164**:709–722.
118. Hahn AB, Soloski MJ. Anti-Qa-2-induced T cell activation: the parameters of activation, the definition of mitogenic and nonmitogenic antibodies, and the differential effects on CD4+ vs CD8+ T cells.
J Immunol 1989;**143**:407–413.
119. Wonigeit K, Schwinzer R. Polyclonal activation of rat T lymphocytes by RT6 alloantisera.
Transplant Proc 1987;**XIX**: 296–299.
120. Kato K, Tamura N, Okumura K, Yagita H. Identification of the T cell surface signal-transducing glycoprotein sgp-60 as CD48, a counter-receptor for mouse CD2.
Eur J Immunol 1993;**23**:1412–1415.
121. Davis LS, Patel SS, Atkinson JP, Lipsky PE. Decay-accelerating factor functions as a signal transducing molecule for human T cells.
J Immunol 1988;**141**:2246–2252.

122. Okada H, et al. 20 KDa homologous restriction factor of complement resembles T cell activating protein.
Biochem Biophys Res Commun 1989;**162**: 1553–1559.
123. Resta R, et al. Glycosyl phosphatidylinositol membrane anchor is not required for T cell activation through CD73.
J Immunol 1994;**153**:1046–1053.
124. Gutensohn W, Resta R, Misumi Y, Ikehara Y, Thompson LF. Ecto-5'-nucleotidase activity is not required for T cell activation through CD73.
Cell Immunol 1995;**161**:213–217.
125. Vogel M, Kowalewski HJ, Zimmermann H, Janetzko A, Margolis RU, Wollny HE. Association of the HNK-1 epitope with 5'-nucleotidase from Torpedo marmorata (electric ray) electric organ.
Biochem J 1991;**278**:199–202.
126. Stochaj U, Dieckhoff J, Mollenhauer J, Cramer M, Mannherz HG. Evidence for the direct interaction of chicken gizzard 5'-nucleotidase with laminin and fibronectin.
Biochim Biophys Acta 1989;**992**:385–392.
127. Dieckhoff J, Mollenhauer J, Kuhl U, Niggemeyer B, von der Mark K, Mannherz HG. The extracellular matrix proteins laminin and fibronectin modify the AMPase activity of 5'-nucleotidase from chicken gizzard smooth muscle.
FEBS Lett 1986;**195**:82–86.
128. Stochaj U, Richter H, Mannherz HG. Chicken gizzard 5'-nucleotidase is a receptor for the extracellular matrix component fibronectin.
Eur J Cell Biol 1990;**51**:335–338.
129. Huizinga TWJ, de Haas M, Kleijer M, Nuijens JH, Roos D, von dem Borne AEG. Soluble Fc-gamma receptor III in human plasma originates from release by neutrophils.
J Clin Invest 1990;**86**:416–423.
130. Shenoy-Scaria AM, Kwong J, Fujita T, Olszowy MW, Shaw AS, Lublin DM. Signal transduction through decay-accelerating factor. Interaction of glycosyl-phosphatidylinositol anchor and protein tyrosine kinases p56lck and p59fyn.
J Immunol 1992;**149**:3535–3541.
131. Su B, Waneck GL, Flavell RA, Bothwell ALM. The glycosyl phosphatidylinositol anchor is critical for Ly-6A/E-mediated T cell activation.
J Cell Biol 1991;**112**:377–384.
132. Robinson PJ, Millrain M, Antoniou J, Simpson E, Mellor AL. A glycophospholipid anchor is required for Qa-2-mediated T cell activation.
Nature 1989;**342**:85–87.
133. Zhang F, et al. Lateral diffusion of membrane-spanning and glycosylphosphatidylinositol-linked proteins: Toward establishing rules governing the lateral mobility of membrane proteins.
J Cell Biol 1991;**115**:75–84.