AMPLIFICATION OF T CELL ACTIVATION INDUCED BY CD73 (ECTO-5'NUCLEOTIDASE) ENGAGEMENT

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In the last years, the surface 69 kD differentiation antigen CD73 has been demonstrated to play a significant role in the activation of human T cells. Unlike other surface molecules involved in T cell activation, CD73 combines three distinct features: i) it has ecto-5'nucleotidase (5'NT) activity (E.C. 3.1.3.5); ii) it is linked to the cell membrane via a glycosyl phosphatidylinositol (GPI) anchor; iii) it can very effectively amplificate T cell activation even if it is expressed by a minority only (15-25%) of T cells.

Role of CD73 in T cell activation

Since CD73 is expected to regulate the uptake of extracellular purines by converting nontransportable nucleotides into readily transportable nucleosides, earlier studies explored the role of the purine salvage pathway in T cell activation. It was shown that the 5'NT activity was crucial for mitogen-stimulated T cells when their capacity to synthetise purine de novo is lost (1). When subsets studies showed that CD73 expression in T cells was mainly confined to CD8+ CD11b- cells containing CTL precursors (2), functional studies were carried out to investigate whether CD73 was involved in CTL generation. These studies used competitive biochemical inhibitors of 5'NT (AOPCP) and cross-reacting antisera (3). The latter suppressed three different steps of cytotoxicity: recognition of stimulatory cells, activation of the cytotoxic program, and binding of target cells. AOPCP only suppressed the second step of cytoxicity, suggesting that inhibition of the enzyme activity was not the only factor involved in the suppression of T cell activation.

Deeper insights into the role of CD73 in T cell activation were obtained when more specific immunologic tools became available. The first was a polyclonal antiserum against human

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CD73 (4). IF studies at the single cell level confirmed the distribution of CD73 in lymphoid subpopulations previously reported by radiochemical and cytochemical assays (4). More importantly, anti-human CD73 serum allowed to purify CD73+ cells and carry on functional analyses (5). It was demonstrated that CD73+ cells have a unique sensitivity to protein kinase C (PKC) activators (5). PKC plays a central role in the cascade of biochemical events initiated by T cell antigen stimulation. Even more interestingly, it was found that CD73 multivalent cross-linking by the polyclonal antiserum delivered activation signals in the presence of submitogenic concentrations of PMA (6). These data demonstrated that CD73 itself could transduce activating signals across the membrane (6). These results were confirmed and extended with monoclonal antibodies (mAbs). So far, at least 5 different mAbs have been produced againts CD73 (1E9, 7G2, 5N2, 27.2, AD2). Functional studies have mostly been carried out with two of them (1E9, 7G2). The latter clearly initiate T cell activation, but they require additional signalling. First, they require cross-linking by a second antibody (6), monocytes (6), or plastic immobilization (7). Second, CD73 engagement requires costimulatory signals like PMA (6) or signals delivered through surface molecules like CD3 and CD2 (7). These data indicate that CD73 itself can transduce activating signals, but this is not enough to fully activate T cells. Rather, CD73 serves as an agonistic molecule to up-regulate T cell activation initiated by antigen recognition (accessory molecule). This function is similar to that of other accessory molecules like CD4, CD8, CD5, CD28, CD44, CD45 etc.. Compared with these molecules, CD73 has some unique features like its enzyme activity and its GPI-linkage to the plasma membrane. Finally, although CD73 is expressed by a minority of T cells and is restricted to a specific subset of CD8+ lymphocytes, its agonistic activity is much more effective than other accessory molecules. CD28 is one of the best characterized accessory molecule which is expressed by more than 70% of T cells. Nevertheless, side by side experiments have shown that CD73 is much more effective than CD28 to promote T cell proliferation induced by CD3 and CD2 stimulations (7). To explain this, it has been proposed that CD73+ cells may act as a specialized subset to amplify immune responses originated by CD3 and CD2 activation pathways: CD73+ cells fully activated by CD3/CD73 costimulation recruit CD73- cells by secreting a number of cytokines (7). Indeed, large amounts of IL-2 are detected in the supernatant of CD3/CD73 costimulated T cells (7). A role of CD73+ cells in the amplification of immune responses is consistent with its decrease in diseases characterized by impaired immune responses (8).

CD73 expression is restricted to naive (CD45RA+) CD8+ cells

In the last years, it has become clear that a major functional distinction among T cells is whether or not they retain the immunological memory of previous antigen encounters. Memory and naive T cells can be discriminated because of a series of phenotypic differences. The most suitable markers to discriminate them are CD45 isoforms (9). Low molecular weight isoforms (CD45R0) are expressed by memory T cells, whereas high molecular weight isoforms are expressed by naive T cells (CD45RA). Beside phenotypic differences, memory and naive cells have different functional properties and activation

requirements. Memory cells (CD45R0+) respond much better to recall antigens or CD3 and CD2 stimulations. Their improved response has been ascribed to several causes: i) different profiles of cytokine production (9); ii) increased expression of accessory molecules (9); iii) pre-assembled organization of the CD3/TCR/CD4/CD8/CD45 transducing apparatus (10). Given the unique ability of CD73 to enhance T cell activation and given its heterogenous expression in CD8+ cells, we have investigated CD73 distribution in purified CD8+ CD45RA+ and CD8+ CD45R0+ cells. These subsets were negatively isolated by using the panning technique. Two sequential pannings with CD4 and CD45RA or CD45R0 mAbs were done. Subsets were 85-95% pure as shown by cytofluorometric analyses. CD73 expression was evaluated by radiochemical and cytofluorometric analyses. Unexpectedly, CD73 expression was found to be confined to naive (CD45RA+) cells (Dianzani et al., manuscript in preparation). This finding pointed out once more the unique characteristics of CD73 as an accessory molecule: so far, CD73 is the only accessory molecule overexpressed in naive rather than memory T cells.

The peculiar expression of CD73 in naive cells may help to revise its role in the immune system and its deficiency in some diseases. One can speculate that its deficiency may reflect either an altered distribution of memory and naive cells or an impaired ability of naive cells to develop an effective immunological memory upon the first encounter with the antigen. In the first case, one should expect an enhanced response to recall antigens and/or CD3 and CD2 stimulations (11); in the latter case, one should find that CD73 has a role in the activation of CD45RA+ cells. Indeed, preliminary studies in our lab indicate that CD73 plays a crucial role in the activation of naive CD8+ cells by making these cells able to overcome their hyporeactivity to CD3 engagement.

CONCLUSIONS

It has been proved that CD73 is an important accessory molecule which can highly upregulate T cell activation. CD73 expression is restricted to a small subset of CD3+ CD8+ CD11b- CD45RA+ cells which are naive T cells containing alloreactive CTL precursors. These data may help to restrict the field on which future studies on CD73 should focus on.

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