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MACROPHAGE AND T LYMPHOCYTE-MEDIATED IMMUNITY: SIMILARITIES AT THE LEVEL OF THE MAC-1 AND LFA-1 MOLECULES

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Introduction

A number of cell surface molecules have recently been characterized which are important in macrophage and lymphocyte cell-mediated immunity. The macrophage Fc receptor [1] and complement receptor type one (CR_1) [2,3] have been characterized by purification and function-inhibiting antibodies. Recently, anti-Mac-1 antibody was found to inhibit the complement receptor type three (CR_3) [4], suggesting that the CR_3 is identical to or associated with the previously biochemically characterized Mac-1 molecule. Studies on T lymphocyte-mediated immunity with function-blocking

antibodies have shown that the Lyt-2,3 and LFA-1 surface molecules are associated with T lymphocyte-mediated killing [5].

Somewhat surprisingly, the Mac-1 molecule associated with CR_3 function, and the LFA-1 molecule associated with T lymphocyte-mediated killing, have been found to be structurally related [6-8]. This suggests that at least with regard to these molecules, similar molecular mechanisms underlie macrophage and T lymphocyte-mediated immunity. Here, the findings on Mac-1 and LFA-1 are reviewed, and the evolutionary implications are discussed.

Mac-1 and complement receptor-mediated adherence

The Mac-1 antigen is expressed on mouse macrophages, blood monocytes, granulocytes, natural killer cells, and 50% of bone marrow cells [9-11]. Mac-1 is absent from lymph node cells, thymocytes, and >90% of spleen cells [9], and thus appears to be a general marker for distinguishing macrophages from lymphocytes [11]. Human Mac-1, defined by cross-reaction of the M1/70 MAb, is present on blood monocytes, granulocytes,

and peripheral blood null cells which contain natural killing and antibody-dependent cytotoxic activity as shown by immunofluorescence cell sorting [12]. The OKM1 [13] and Mo1 [14] antigens have the same distribution, and may be identical to human Mac-1.

To determine whether Mac-1 was related to any of the known, functionally important receptors on the macrophage surface, the anti-Mac-1 MAb was tested for inhibition of Fc and complement receptors [4]. Addition of anti-Mac-1 MAb to rosetting assays decreased the percentage of macrophages rosetting with E-IgM-C (erythrocyte-IgM antibody-complement complexes from control levels of 96% to 12%. In contrast, MAb binding to six other types of antigens on macrophages had no effect on E-IgM-C rosetting. None of the above MAb significantly inhibited macrophage Fc receptor-mediated rosetting of E-IgG. Furthermore, anti-Mac-1 $F(ab')_2$ fragments at concentrations as low as 1 μg/ml strongly inhibited rosetting of E-IgM-C but had no effect on rosetting of E-IgG. As expected from its cross-reactivity with human cells, anti-Mac-1 MAb was

also found to inhibit E-IgM-C rosetting by human polymorphonuclear leukocytes (PMNL). Macrophages and PMNL express two types of complement receptors, CR_1 specific for C3b, and CR_3 specific for C3bi and possibly its further degradation products α2D and C3d,g [15,16]. CR_2 which is specific for C3d is absent from myeloid cells, but is expressed on B lymphocytes. The specificity of the complement receptor inhibited by anti-Mac-1 was examined by using E coated with purified complement components C3b as a probe for the CR_1, or C3bi as a probe for the CR_3. Anti-Mac-1 blocked CR_3 on both human granulocytes and mouse macrophages, but CR_1 on neither cell. The most likely interpretation of these results is that Mac-1 antigen is the CR_3. However, the possibility remains that Mac-1 is a distinct moiety which is closely associated with CR_3 or promotes its function.

LFA-1, a Mac-1 homologue which participates in immune T cell interactions

In separate but parallel work on the molecular basis of T cell function, the LFA-1 molecule was

discovered. This section will summarize the functional data on LFA-1, while the following section will describe how LFA-1 and Mac-1 are structurally related. To identify molecules associated with antigen-specific T lymphocyte-mediated killing of target cells, MAb were prepared and screened for their ability to inhibit killing [17-21]. MAb to two types of antigens, LFA-1 and Lyt-2,3, were found to consistently inhibit killing. In contrast, MAb to at least ten other types of lymphocyte cell surface antigens, some of which bind to a higher number of sites per cell, were found to have little effect on killing. Inhibition by anti-LFA-1 MA is not due to trivial causes such as toxicity or agglutination. Inhibition is due to binding of MAb to the CTL effector rather than to the target cell. The simplest explanation of these results is that the LFA- molecule plays an important role in the interaction between cytolytic T lymphocytes and target cells.

CTL-mediated killing involves three steps: Mg^{+2}-dependent and specific antigen recognition-dependent adhesion of the CTL to the target cell,

Ca^{+2}-dependent delivery of the lethal hit to the target cell, and CTL-independent target cell lysis. As shown by microscopic enumeration of CTL-target conjugates, anti-LFA-1 MAb block the adhesion step [18]. If CTL and target cells are incubated in the presence of magnesium but absence of calcium, allowing adhesion formation but not lethal hit delivery, addition of anti-LFA-1 MAb reverses preformed CTL-target conjugates and inhibits the killing of targets which occurs after calcium is added [21]. If addition of anti-LFA-1 MAb is delayed until after the addition of calcium, there is no inhibition. Anti-LFA-1 MAb inhibits lectin-dependent killing at low but not high lectin concentrations [21]. These results suggest that LFA-1 contributes to the formation of the adhesion between the CTL and target cell.

LFA-1 has also been found to be important in killing by CTL-T lymphoma hybridomas. Several CTL hybridomas have been obtained which show highly specific killing directed to $H\text{-}2^b$ target cells [22]. Interestingly, the hybridomas express LFA-1 but not Lyt-2,3, as

shown with a number of MAb specific for topographically distinct determinants on these molecules [23]. Furthermore, the anti-LFA-1 MAb completely abolish cytolysis mediated by the CTL hybridomas, but anti-Lyt-2,3 MAb have no effect.

Anti-LFA-1 MAb also inhibit the induction of antigen-specific T helper lymphocyte proliferative responses, and the mixed lymphocyte reaction [18].

The structural relationship between Mac-1 and LFA-1

In overall structure the Mac-1 and LFA-1 antigens are very similar [6,7]. Mac-1 contains α and β subunits of 170,000 and 95,000 M_r, respectively. Crosslinking of the detergent solubilized Mac-1 molecule has shown that the subunits are noncovalently associated in an $\alpha_1\beta_1$ structure. The LFA-1 antigen contains α and β subunits of 180,000 and 95,000 M_r which are noncovalently associated in the same type of $\alpha_1\beta_1$ structure. In Mac-1 and LFA-1, the α and β subunits are labeled by ^{125}I, [^{35}S]methionine, and [^{3}H]glucosamine in intact cells, showing both chains are glycoproteins with sur-

face exposure and are synthesized by the cells on which they are expressed. Three independent MAb obtained to Mac-1 do not crossreact with LFA-1 and five independent MAb to LFA-1 do not crossreact with Mac-1 as shown by immunoprecipitation [21]. This demonstrates that Mac-1 and LFA-1 contain unique antigenic determinants. One MAb, M18/2, crossreacts between LFA-1 and Mac-1 [21], demonstrating common determinants. Conventional antisera to purified Mac-1 and LFA-1 also crossreact. Immunoblotting experiments have been conducted by separating the α and β subunits by SDS-PAGE, electrophoretically transferring the proteins to nitrocellulose, and probing with monoclonal or conventional antibodies followed by ^{125}I-labeled second antibodies [24]. It was found that the M18/2 MAb reacts with the Mac-1 and LFA-1 β subunits. Conventional antisera to Mac-1 react strongly with the Mac-1 and LFA-1 β subunits, and with the Mac-1 but not the LFA-1 α subunit. These results suggest that antibodies crossreacting between Mac-1 and LFA-1 react with determinants on the β chains. Thus far, no reactivity of the non-

crossreactive MAb has been obtained in immunoblotting experiments, suggesting these MAb react with conformational or combinatorial determinants which are not retained after α and β subunit separation in SDS-PAGE.

The structural basis of the relationship between LFA-1 and Mac-1 has been investigated by peptide mapping. Affinity-purified LFA-1 and Mac-1 antigens [7] were iodinated, the α and β subunits separated by SDS-PAGE, and subjected to peptide mapping [6]. The LFA-1 and Mac-1 β subunits share at least 10 tyrosyl tryptic peptides and thus are highly homologous or identical. In contrast, the Mac-1 and LFA-1 α subunits show at least 17 unique tryptic peptides each. The extensive differences strongly suggest that the Mac-1 and LFA-1 α chains are products of distinct genes. However, since only one amino acid substitution per tryptic peptide would generate different maps, it cannot be ruled out that the α subunits are homologous. This will have to be determined by amino acid sequencing studies. The fact that both α subunits bind to a

common or homologous β subunit suggests that they have common structural features and hence sequence homology.

To determine whether the Mac-1 α and β subunits are derived from a common or separate precursors, their biosynthesis has been examined [25]. The Mac-1 α and β subunits are derived from precursors of 161,000 and 87,000 M_r, respectively. The precursors were found to be synthesized as free chains which became associated into the $\alpha_1\beta_1$ complex and then processed to the slightly higher M_r of the mature subunits. The results suggest that α and β are synthesized from separate mRNA transcripts and hence from separate genes.

Mac-1 and LFA-1 comprise a novel family of leukocyte differentiation antigens in which alternative forms of an α subunit of 170,000 to 180,000 M_r are noncovalently associated with a common or highly homologous β subunit of 95,000 M_r in $\alpha_1\beta_1$ structures. The large structural differences in the α subunit suggest they bear the unique MAb-defined epitopes. Common determinants recognized by conventional antisera to purified Mac-1 and LFA-1 and by the M18/2 MAb are present on the β subunit. The α subunits appear specialized, perhaps for binding of different ligands, while the β subunits would be expected to perform a common function, as in regulation or signal transduction. The separate synthesis of the α and β subunits followed by their association into $\alpha_1\beta_1$ structures provides a mechanism whereby a single β subunit could be used for the synthesis of both Mac-1 and LFA-1. The selective expression of the Mac-1 and LFA-1 α chains in the monocytic and lymphoid lineages shows that α gene expression is closely coordinated with leukocyte differentiation. An exciting prospect concerning LFA-1 and Mac-1 is that since the antigens are structurally related, and both are function-associated detailed structure-function comparisons are feasible.

Speculations on the evolutionary origin of Mac-1 and LFA-1

Despite the differences in function between Mac-1 and LFA-1, there are also common features. Both molecules act to strengthen adhesions between effector cells and target cells. Mac-1 functions in nonspecific immunity either as the complement receptor type 3 or to promote the activity of this receptor.

While LFA-1 functions in specific immunity, its role in promoting T cell adhesions appears non-antigen-specific. Both in the specific and nonspecific immune functions in which LFA-1 and Mac-1 participate, respectively, it appears that the participation of additional cell surface molecules is required for optimal functional activity. LFA-1 may be only one of several receptors including Lyt-2,3 and the antigen receptor which mediate T cell interactions [21]. The complement receptor type 3 acts synergistically with other cell surface receptors such as the Fc receptor to stimulate phagocytosis by macrophages and granulocytes [26] and killing by antibody-dependent cytotoxic effectors [27].

Although there is as yet no direct evidence for homology between the Mac-1 and LFA-1 α subunits, general knowledge of the chemistry of multi-subunit protein families suggests this is almost certainly the case. There are many families in which a common subunit can be associated with alternative forms of a second subunit. Examples are the hemoglobins [28] and the class I histocompatibility antigens [29]. By analogy to these families, it would be predicted that the Mac-1 and LFA-1 α subunits are homologous, that they arose by gene duplication, and that they may have remained closely linked genetically.

How might have this protein family evolved, in which at least two members, Mac-1 and LFA-1, mediate distinct cell surface functions on distinct cell lineages? One possibility would be that members of this family mediate cell interactions on many different cell types, and have evolved to mediate different functions on each different cell type. Thus, homologues of LFA-1 and Mac-1 might be found on brain cells, fibroblasts, endothelial cells, and so on. However, attempts to isolate LFA-1 or Mac-1-like molecules from non-hematopoietic tissues using the anti-β subunit M18/2 MAb have thus far been unsuccessful. Therefore, the idea is favored that the protein ancestral to LFA-1 and Mac-1 arose within the host defense system. It is hypothesized that this ancestor functioned in the host defense system which predated the acquisition of specific immunity during vertebrate evolution. These speculations are outlined in Figure 1. In mammals

today, the alternative complement pathway is activated in an antigen-nonspecific, antibody-independent fashion by foreign organisms such as yeast. This results in the deposition of fragments of C3, opsonizing the particles for phagocytosis through C3 receptors. Nonspecific immunity mediated by invertebrate phagocytes involves the recognition and ingestion of opsonized foreign particles [30], and may utilize proteins very similar or homologous to those of the alternative complement pathway. Molecules associated with complement receptor activity such as Mac-1 thus probably evolved before specific immunity. Accompanying the development of specific immunity was the divergence of cells mediating specific immune functions (lymphocytes) and those mediating nonspecific immune functions (macrophages, granulocytes, and large granular lymphocytes). It is speculated that before or at this time the genes for the α subunits duplicated and diverged to mediate specialized functions in these distinct cell lineages

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FIG. 1. Speculations on the evolutionary divergence of LFA-1 and Mac-1 α subunits.

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DIFFERENTIATION ANTIGENS ON HUMAN MONOCYTES AND MACROPHAGES DEFINED BY MONOCLONAL ANTIBODIES

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INTRODUCTION

We have recently developed a panel of hybridoma-derived mouse monoclonal antibodies that are reactive with human monocyte-myeloid cell antigens (1,2). Among human peripheral blood (PB) elements, anti-Mol detects an antigen expressed by monocytes, granulocytes and a subset of sheep erythrocyte rosette negative (E-), surface immunoglobulin negative (sIg-) null cells. Anti-Mo2 binds selectively to PB monocytes. Anti-Mo3 is specific for an antigen that is weakly expressed by circulating monocytes but is strongly expressed by cultured monocytes and macrophages. Anti-Mo4 detects an antigen that is co-expressed by most monocytes and all platelets. Anti-Mo5 binds to an antigen found on monocytes and granulocytes, but unlike Mol, is not expressed by null cells. Anti-Plt-1 is specific for an antigen that is selectively expressed by platelets. In this report, we will review our progress in the further characterization of the antigens defined by these monoclonal reagents and will examine their expression during myeloid-monocyte differentiation.

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