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12

Inherited LFA-1, Mac-1 deficiency and its molecular biology

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The LFA-1, Mac-1 glycoprotein family

A family of functionally important, high molecular weight glycoproteins, i.e., the macrophage differentiation antigen (Mac-1), the lymphocyte-function assiociated antigen (LFA-1), and the p150,95 molecule, with identical β subunits but different α subunits has recently been defined on leukocyte cell surfaces in the mouse (1) and human (2). The properties of the Mac-1, LFA-1, and p150,95 family are summarized in Table 1 (1–12). The LFA-1 and Mac-1 molecules function in differing types of leukocyte adhesion reactions.

Anti-LFA-1 monoclonal antibody (MAb) inhibit the first step in T cell-mediated killing, i.e. the adherence of the killer cell to the target cell (3, 13, 14). Non-antigen-specific natural killing (6, 15, 16), and antibody-dependent cellular cytotoxicity (17, 18) are also inhibited. The importance of LFA-1 in leukocyte adhesion is not limited to immune interactions. LFA-1 MAb inhibit spontaneous (5, 19) and phorbol ester induced (20) homotypic aggregation of B and T cell lymphoblastoid lines. The findings suggest LFA-1 acts as a cell adhesion molecule which can synergize with antigen-specific, Fc, or other receptors to increase the avidity of cell-cell interactions.

Anti-Mac-1 MAb inhibit the complement receptor type 3 (CR3) on mouse and human cells (10). The CR3 mediates adherence and phagocytosis of particles opsonized with iC3b, a cleaved, hemolytically inactive form of the third component of complement. MAb to the Mo1, OKM10, and in some cases the OKM1 determinants on the human Mac-1 molecule also inhibit CR3 function (2, 11, 12). MAb which bind to other determinants on Mac-1 but do not inhibit CR3 activity have also been obtained (1, 12). Such MAb-Mac-1 antigen complexes bound to S. aureus bacteria specifically agglutinate iC3b opsonized erythrocytes (12), demonstrating the the Mac-1 molecule has CR3 activity.

The members of the LFA-1, Mac-1 glycoprotein family contain identical β subunits noncovalently associated with different α subunits (Table I). Crosslinking experiments have shown the quaternary structure of each antigen is $\alpha_1\beta_1$ (2, 21). The β subunits are identical by peptide mapping (22, 23), in isoelectric focussing (2), and by complete immunological crossreactivity (1). The α subunits differ by the same criteria.

Studies of dissociated subunits have clarified both MAb specificity and the interrelationships of this glycoprotein family in mouse and man (1, 2). Antibodies specific for LFA-1 react with the αL subunit; those specific for Mac-1 react with αM. Antibodies to the shared β subunit react with LFA-1, Mac-1, and a third member of the family, p150,95 molecule. p150,95 contains the αX subunit for which specific MAb have not yet been obtained.

The N-terminal amino acid sequences of the murine Mac-1 and LFA-1 α subunits show 33 per cent identity (24). This homology suggests their genes arose by a duplication event. The identity between the β subunits suggests that the divergence between the α subunits confers adhesive specificity. Blocking experiments with subunit-specific MAb are in accord with this idea (1, 2, 12).

Table I. The LFA-1, Mac-1 family.*

Mouse	LFA-1	Mac-1	
Human	LFA-1	Mac-1 (OKM1, Mo1)	p150,95
Subunits**	αL β	αM β	αX β
$M_r \times 10^{-3}$	180,95	170,95	150,95
Function	Adhesion	Complement receptor type 3	?
Cell distribution	Lymphocytes	Monocytes	
	Monocytes***	Macrophages	Monocytes
	Granulocytes	Granulocytes	Granulocytes
	LGL	LGL	Others?

* From references 1–12.

** Noncovalent $\alpha_1\beta_1$ association, β's are identical.

*** Low expression on blood monocytes and some macrophage cell lines, absent from thioglycollate-elicited macrophages

Deficiency of an entire surface glycoprotein family

A clinical syndrome characterized by recurrent bacterial or fungal infection, progressive periodontitis, persistent leukocytosis, and/or delayed umbilical cord separation has been described in patients whose neutrophils demonstrated severely depressed adherence, chemotaxis, and phagocytic function (25–28). When total, surface-labeled granulocyte proteins from these patients were subjected to SDS-PAGE, a lack was noted in a glycoprotein(s) variously estimated to be M_r = 110 000 (25), 180 000 (26), 150 000 (27), or 138 000 (28). Deficiency of a specific surface molecule in one of these patients was pinpointed in December, 1981 using ^{125}I-labeled anti-Mac-1 MAb (Table II). The Mac-1 α subunit is present in only about 2 per cent of normal amounts on the patient's cells. Subsequently, deficiency in the Mac-1 α subunit was also found with the Mo1 and OKM1 MAb, and deficiency in the Mac-1 β subunit was detected with the TS1/18 MAb (28, 29).

The deficiency of both the α and β subunits of Mac-1 suggested that other members of the LFA-1, Mac-1 glycoprotein family might also be deficient. Here we report studies on the molecular characterization of the defect in three unrelated patients and their kindred from the Houston and Galveston, Texas, referral areas.

MAb specific for the αL subunit of LFA-1, the αM subunit of Mac-1, and the common β subunit were used to quantitate surface expression by immunofluorescent flow cytometry (data not shown). Patient granulocytes and mononuclear cells were markedly deficient in all three subunits. An interesting quantitative difference was seen between patients. Patients 1 and 2 expressed between 0.2 to 0.5 per cent of the normal amounts of the three

Table II. Anti-Mac-I^{125} I-MAb binding to normal and patient granulocytes.*

Cells	Preincubation with unlabeled MAb	^{125}I-Anti-Mac-1 (cpm bound)	Specific binding	Binding (% normal)
Patient	Control	256	48	2
Patient	Anti-Mac-1	208		
Control	Control	2611	2348	(100)
Control	Anti-Mac-1	263		

* Granulocytes (6 × 10 in 50 μl) were preincubated with 20 μl of 100 μG/ml M1/70 anti-Mac-1 MAb or control M1/69HK MAb, then ^{125}I-labeled M1/70 was added. After ½ hour at 4° C, cells were washed and γ-counted. Patients' granulocytes were provided by Drs. A. Arnaout and J. Pitt.

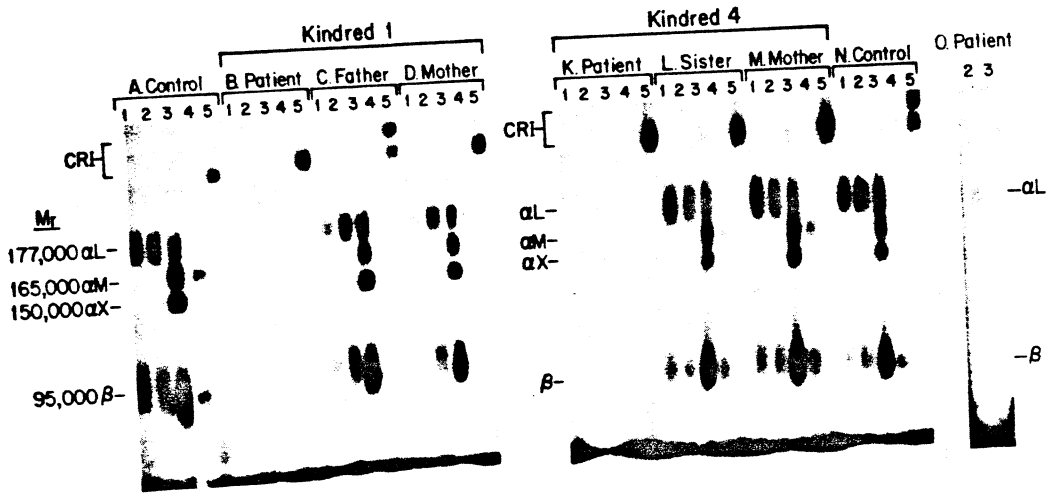

Fig. 1. Immunoprecipitation of ^{125}I-labeled surface proteins from granulocytes. Granulocytes were surface-labeled with ^{125}I using lactoperoxidase. Triton X-100 lysates were immunoprecipitated with a mixture of five different anti-LFA-1 α MAb (lane 1), anti-LFA-1 α TS1/22 MAb/Sepharose CL-4B (lane 2), anti-β TS1/18 MAb-Sepharose (lane 3), anti-OKM1 MAb (lane 4), or anti-CR1 44D MAb (kindly provided by Dr. V. Nussenzweig) (lane 5). Immunoprecipitates for lanes 1, 4, and 5 were formed with anti-mouse IgG and S. aureus. Precipitates were subjected to SDS 7 per cent PAGE and autoradiography.

subunits, values that are marginally distinguishable from complete absence. In contrast, patient 4 expressed clearly higher but subnormal amounts of 3, 9, and 7 per cent of the αL, αM, and β subunits, respectively. Incubation of normal granulocytes with 10^{-8} M f-Met-Leu-Phe resulted in about 1.3-fold, 5.5-fold, and 3.7-fold upregulation in the amount of surface accessible αL, αM, and β, respectively. However, expression on cells of patients 1 and 2 showed little if any increase. Deficiency was seen with MAb to four and three different topographic sites on the αL and αM subunits, respectively. In contrast, the complement receptor type I (CRI), neutrophil FcR, and HLA antigens were present in normal amounts. Mothers and fathers of the three patients had 53 to 63 per cent of normal amounts of the subunits on the cell surface. Together with uniform expression on mothers' granulocytes demonstrating no mosaicism due to X chromosome inactivation, and a fourth family with an affected father, son, and daughter, these findings show somatic recessive inheritance.

To examine the deficiency at a molecular level, patients' granulocytes were labeled with ^{125}I and subjected to immunoprecipitation. Representative experiments with kindreds 1 and 4 are shown in Figure 1. From control cells, the anti-αL MAb precipitated the LFA-1 αL subunit noncovalently associated with its β subunit (Fig. 1A, lanes 1 and 2). Anti-αM MAb precipitated the αM subunit with its β subunit (Fig. 1A, lane 4). The anti-β MAb precipitated the β subunit and the three types of a subunits noncovalently associated with it: αL, αM, and the αX subunit of the p150,95 molecule (Fig. 1A, lane 3). In contrast, cells from patients were strikingly deficient in all these molecules (Fig. 1B and K, lanes 1–4). The normal parents and siblings of the patients were positive for immunoprecipitation. The CRI was present in similar amounts in normal and patient cells (Fig. 1 A–N, lane 5).

To test for low levels of expression in patient cells, autoradiograms were subjected to prolonged exposure. Precipitation of small amounts of LFA-1 were detected in patient 4 (Fig. 4, lanes 2–3) but not in patients 1 and 2. Notably, anti-αL and anti-β MAb each precipitated both α and β subunits. This shows that when surface expression can be detected on patient cells, it is due to the presence of the normal αβ complex, rather than to the presence of free α or β subunits.

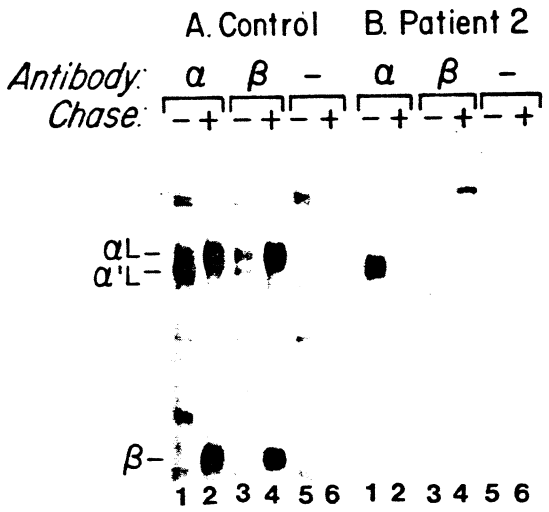

Fig. 2. LFA-1 biosynthesis in patient and normal cells. PHA blasts were labeled with [^{35}S] methionine for two hours, and either harvested immediately or chased for 22 hours with unlabeled methionine as indicated. Lysates with Triton X-100 and sodium deoxycholate were immunoprecipitated with purified antibodies coupled to Sepharose CL-4B (2): TS1/22 anti-LFA-1 α, TS-1/18 anti-β, or activated, quenched sepharose as negative control. Precipitates were subjected to SDS-PAGE and fluorography.

Since the Mac-1, LFA-1, and p150,95 molecules utilize identical β subunits, the lack of this entire glycoprotein family suggested that the primary defect might be in the β subunit. Biosynthesis experiments in normal cells have shown that the α and β subunits are derived from separate precursors denoted α′ and β′, and that association into an α′β′ complex precedes processing to the mature αβ form and expression on the cell surface (2, 8).

To examine biosynthesis, cells were pulsed with [^{35}S] methionine to label precursors, and then either examined immediately or chased with cold methionine to follow processing and maturation. Figure 2 shows results with patient 2 PHA blasts; identical results were obtained with EBV lines of patients 1 and 4. In normal cells, α′L was synthesized (Fig. 2A, lane 1), and after the chase, was converted to the higher M_r αL form (Fig. 2A, lanes 2 and 4), which was associated with β (Fig. 2A, lanes 2 and 4). Subunit association preceded processing, as shown by precipitation of α′L with anti-β (Fig. 2A, lane 3). In patients' cells, the α′L precursor was found but it never matured (Fig. 2B, lanes 1 and 2), and lack of precipitation of α′L by anti-β MAb showed it did not become β associated (Fig. 2B, lanes 3 and 4). Patient and control α′L were present in similar amounts after the two hour pulse, suggesting they have equal stability. As expected, the α′L precursor was degraded in patients' cells after the 22 hour chase, showing intracellular, unassociated α′L has a shorter halflife than extracellular, associated αL (Fig. 2B, lane 2 compared to Fig. 2A, lane 2).

The findings on the molecular basis of the defect in patients' cells, and previous work on normal cells, are summarized in Figure 3. The primary deficiency in patients' cells appears to be in the β subunit. The presence of α′L precursor but no αL inside patients' cells, and the absence of cell surface

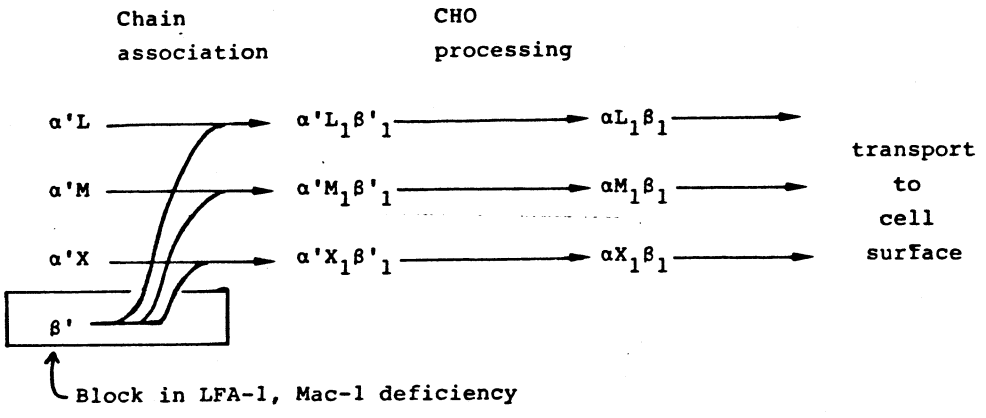

Fig. 3. Biosynthesis of the LFA-1, Mac-1 Family.

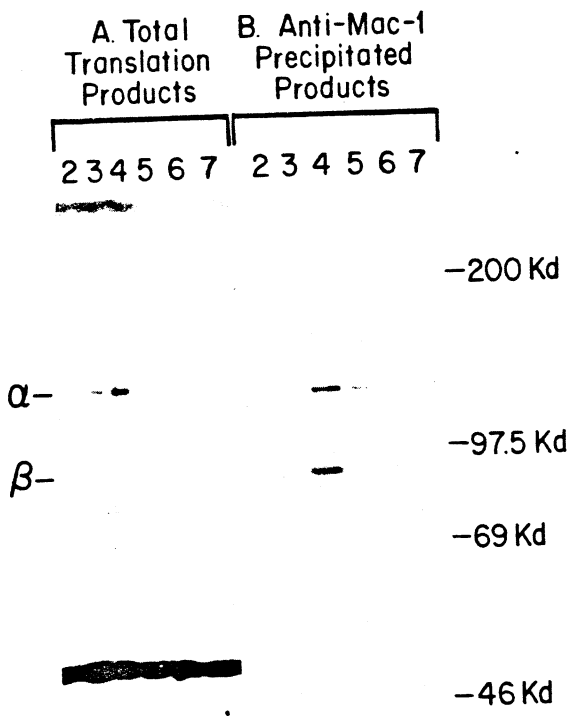

Fig. 4. Translation products of immunoselected Mac-1 polysomes. P388D$_1$ polysomes were incubated with rabbit anti-Mac-1 IgG and specific Mac-1 polysomes were bound to a protein A-Sepharose column as described by Shapiro and Young (36). The mRNA from the immunoselected polysomes was eluted with 20 mM EDTA; 400 μl fractions were collected and ethanol precipitated. One fourth of the mRNA in each fraction was collected by centrifugation and translated in vitro using a rabbit reticulocyte system. The products of translation were analyzed by SDS-7.5 per cent PAGE and fluorography. Panel A shows the polypeptides present in 1 μl of total in vitro translation products from eluted fractions 2 to 7. Panel B shows the polypeptides that were immunoprecipitated from 24 μl of translation products with rabbit anti-Mac-1 serum by the method of Anderson and Blobel (37). The positions of the α and β precursors synthesized in vitro from total P388D$_1$ mRNA and identified by immunoprecipitation are indicated.

αL, show that subunit association is required for processing and transport to the surface. Thus, complementation between α and β for surface expression reflects the requirement for αβ subunit association. Biosynthesis experiments have been done thus far in cells which only express LFA-1, but the similar biosynthetic pathways of Mac-1 and p150,95 in normal cells (2) suggests this finding can be generalized as indicated in Figure 3. The finding of small amounts of the αβ complex on the surface of patient 4's cells suggests that only a small amount of β is made, which limits surface expression of the complex. This may be a different mutation than that in patients 1 and 2.

A similar molecular deficiency in other patients throughout the world is being seen by immunofluorescence and in a few cases by immunoprecipitation of surface molecules (28, 30–33). In all cases a lack of the LFA-1 and Mac-1 α subunits and the common β subunit is being found. Heterogeneity among these patients in their symptoms heretofore hindered their classification into a single clinical entity. A common molecular basis for their disease has now been demonstrated: inherited LFA-1, Mac-1 deficiency.

This deficiency disease has a dramatic impact on leukocyte function. Granulocytes appear to be the most severely affected cell type, perhaps because they normally express all three members of this glycoprotein family. Granulocytes from patients studied here are deficient in the Rebuck skin window test, baseline and stimulated adherence to surfaces, spreading, and aggregation (28, 34). Orientation and chemotaxis are affected, probably secondary to failure to attach normally. Granulocyte antibody-dependent cellular cytotoxicity and mononuclear cell natural killing are also affected (17). T lymphocytes which normally express LFA-1 show lower responses to mitogens and lower cell-mediated killing (20). These findings demonstrate the importance of this glycoprotein family in cell adhesion, and are in excellent agreement with previous MAb blocking studies.

An interesting difference between patients has been noted in CR3 function. An early study found normal CR3 on patients' cells as measured by iC3b rosetting, and concluded that the CR3 was distinct from Mo1 (35). However, further study on this patient's cells showed expression of 7 per cent of normal levels of Mo1 (= Mac-1) and inhibition of CR3 function with anti-Mo1 MAb (29). In contrast, patient 1 of this study, expressing 0.3 per cent of Mac-1, is completely negative for the CR3 (28). The differences in CR3 assays therefore appear to reflect differing levels of Mac-1 expression.

Mac-1 mRNA purification and translation

Mac-1 cDNA clones would be invaluable for analyzing the genetic basis of the defect in patients' cells, the complete amino acid sequence of the α and β subunits, the genetic organization of the protein family, and the regulation of gene expression in differentiation. As an important step towards this goal, Mac-1 mRNA has been isolated and its in vitro translation products identified. A rabbit antiserum was prepared against mouse Mac-1 antigen purified on MAb-Sepharose affinity columns (21). IgG was purified and freed of RNAse on protein A-Sepharose. Polysomes from the $P388D_1$ macrophage-like line were reacted with anti-Mac-1 IgG. After preclearing by passage through an activated, quenched Sepharose 4B column, specific polysomes bearing nascent Mac-1 α or β chains to which anti-Mac-1 IgG was bound were isolated by passage through Protein A-Sepharose. After washing, mRNA was eluted from the Ab-nascent chain-ribosome-mRNA complex with EDTA. Fractions were translated using a rabbit reticulocyte lysate system in the presence of [^{35}S] methionine and subjected to SDS-PAGE. The major translation product was the Mac-1 α subunit (Fig. 4A). Its identity was confirmed by immunoprecipitation with anti-Mac-1 serum, which also detected by β subunit (Fig. 4B). Since the Mac-1 molecule represents only 0.1 per cent of $P388D_1$ cellular protein (21), polysome immunoselection achieved a remarkable purification of specific mRNA. This mRNA is being used for the isolation of Mac-1 cDNA clones.

Summary

The molecular and functional properties of a family of leukocyte surface molecules with different α subunits and a common β subunit are summarized. The entire family of proteins is deficient on the surface of leukocytes of patients with recurring, life-threatening infections. This novel immunodeficiency disease demonstrates autosomal, recessive inheritance. Patient cells have normal amounts of intracellular LFA-1 α precursor, but the α subunit precursor does not mature or become surface expressed. Together with surface deficiency of all molecules with the common β subunit, this suggests that 1) the primary deficiency is of the β subunit, and 2) association of α with β is required for maturation and transport to the cell surface. The molecular biology of this protein family and its deficiency is beginning to be studied. Messenger RNA of the murine Mac-1 α and β subunits has been purified by isolation of nascent polypeptide chain-polyribosome complexes with polyvalent antibody to Mac-1. The Mac-1 subunits are the predominant translation products of the purified mRNA, and their authenticity was established by immunoprecipitation.

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Discussion

Edited by R.M. Steinman and H.L. Langevoort

Bianco: How did you manage to precipitate both α and β chains from your polysomes?
Springer: Our antibody was a rabbit Ig prepared to α–β dimer.
Cohn: What is the topography of these molecules on the cell surface?
Springer: Both chains have carbohydrates and are exposed externally. Papain can cleave the α and β subunits, but the molecules remain associated with each other and the cell surface. Each chain may transverse the membrane and more than once, but we know little about cytoplasmic domains.
Mellman: Do you think the β subunit is needed to transport the α chain to the cell surface?
Springer: Yes. We know that α and β are associated prior to transport to the cell surface. As I described, some Mo-1 deficient patients make the α chain but are not inserted in the cell surface.
Schreiber: The collaboration with a Seattle group, we have identified a monoclonal 60.3 that appears to be to the β chain and does inhibit function.
Nussenzweig: Do the parents of the patient exhibit decreased leukocyte adhesiveness?
Springer: The parents appear normal.
Nussenzweig: Do antibodies to the α chain interfere with adhesion of normal cells?
Springer: We have just been doing these experiments. The preliminary data are that mixtures of antibodies can alter adhesion.
Gallin: Can you put the Mac-1 molecule in liposomes and restore function to Mo-1 deficient cells?
Springer: I think it would be hard to cure a patient that way. Actually, we are waiting for genomic α and β DNA so that we could do the restoration experiment by transfection.
Steinman: LFA-1 is involved in CTL-target interaction. What is known about the site recognized by LFA-1 on the target?
Springer: We have evidence that the 60 Kd LFA-3 on the target cell is required, but we have little additional information on the ligand for LFA-1. It will be a difficult problem experimentally if the ligand-LFA-1 affinity is as low as the monomeric C3bi-Mac-1 affinity.
Wright: I have a monoclonal 1B4 that competes with the Seattle antibody, 60.3. It does not block phagocytosis of C3bi coated erythrocytes when used as a Fab, but inhibits by 50 per cent as intact immunoglobulin. We have also tested α-LFA-1, OKM1 and α–β chain antibodies as inhibitors of leukocyte migration across endothelia, but there was no block.
Gordon: Is there any defect in Fc receptor function, as in ADCC?
Springer:Fc receptor function on the patient's cells is normal, i.e., rosetting with antibody coated red cells, and spreading on IgG coated-plates. However, the patients are deficient in ADCC to antibody-coated tumor cells and red cells. This seems surprising at first. However, one might consider the fact that T cell recognition of antigen can be influenced by molecules like T8 which are not thought to be part of the specific antigen receptor.