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Chapter 35

Human Cytolytic T-Lymphocyte Clones and Their Function-Associated Cell Surface Molecules

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1. Introduction

Cytolytic T lymphocytes (CTLs) are important effectors in the recognition of viruses,[1] allografts,[2] and some tumors.[3] A molecular understanding of the CTL–target cell interaction therefore may be relevant to the etiology and/or treatment of a variety of disease states. Tissue culture techniques, first reported by Gillis and Smith,[4] have allowed the generation of long-term T-cell lines that retain function. A number of human long-term cytolytic T lymphocyte lines have been generated by continued stimulation of peripheral blood lymphocyte lines with "foreign" cells in the presence of the T-cell growth factor interleukin 2 (IL-2).[5,6] We have used CTL lines and clones to define target antigens recognized by human allogeneic lymphocytes, to correlate lymphocyte phenotype with antigen specificity, and to generate monoclonal antibodies that block lymphocyte function. Our findings have provided new insights into the cell surface molecules involved in the CTL–target cell interaction. In this chapter we describe our methodologies for the generation and maintenance of CTL lines and

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clones and the use of these cells in concert with monoclonal antibodies to define and analyze function-associated cell surface molecules.

2. Methods

2.1. Generation of T-Cell Growth Factors (Conditioned Medium)

Although interleukin 2 (IL-2) is requisite for the proliferation and maintenance of human T cells, it may not be sufficient for the long-term maintenance and cloning of CTLs. We have found that IL-2 produced by the T-cell tumor Jurkat or by recombinant techniques support short-term CTL growth and proliferation but poorly maintains long-term growth or the cloning of human CTLs. These findings suggest that other, as yet undefined factors may also be required for optimal *in vitro* growth of human CTLs. We therefore have used conditioned medium, i.e., supernatants from lectin- and allostimulated peripheral blood lymphocytes, as our source of T-cell growth factors (TCGFs) necessary for the long-term maintenance of CTL lines and clones.

Peripheral blood lymphocytes (PBLs) are obtained from plasmapheresis donors with approximately (1–5) $\times$ 10^9 mononuclear cells obtained per donor. Cells from five different donors are pooled to minimize the possibility of "low IL-2-producers" as well as to maximize allostimulation. The cells are washed three times and cultured at 3 $\times$ 10^6 cells/ml. It is not necessary to remove contaminating erythrocytes.

We add mitogen and several cell cycle inhibitors to maximize TCGF yield. PHA-P (Difco) (0.15% v/v) is preferable to Con A or LPS in stimulating TCGF production by human PBLs. In addition, 2.8 μM indomethacin (Sigma) is added to inhibit prostaglandin synthesis by adherent cells. Hydroxyurea (50 μM) is added to prevent cells from entering S phase and thereby minimizes the absorption of IL-2 by proliferating cells (interleukin 2 is produced during late G_1).[7]

Cells are cultured in RPMI 1640 (M.A. Bioproducts) supplemented with 3% fetal calf serum, 2 mM L-glutamine (GIBCO), penicillin at 100 U/ml and streptomycin at 100 μg/ml (GIBCO), and 10 mM HEPES (M.A. Bioproducts) in 150-cm^2 flasks at 37°C in a 5% CO_2 humidified incubator for 72 hr. The supernatants are harvested and PHA and other additives are depleted by sequential precipitations at 50 and 75% ammonium sulfate saturation.[8] The precipitate at 50% saturation is discarded, and the precipitate at 75% saturation is suspended in one-tenth the original volume of RPMI 1640, and dialyzed against five changes of PBS and a final change of RPMI 1640. The final preparation is filtered and stored at 4°C or aliquoted and frozen at −20°C. The activity of each preparation is assessed by measuring proliferation ([^{3}H]thymidine incorporation) of an IL-2-dependent T-cell line such as HT-2.

2.2. Generation of CTL Lines and Clones

Cytolytic T lymphocytes (CTLs) are generated by stimulating PBLs with an Epstein–Barr virus (EBV)-transformed B-cell tumor of known HLA type. In

general, 5×10^6 PBLs/ml are stimulated with 1×10^5 irradiated (10,000 rad) stimulator cells in 2-ml wells (Linbro). Cultures are restimulated at 7- to 10-day intervals and are cloned by limiting dilution at various times after primary stimulation. Limiting dilution methodology and theory have been described in detail elsewhere.[9] Cells are cloned in RPMI 1640, supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin 100 U/ml, streptomycin 100 μg/ml, 25 μM 2-mercaptoethanol, and 10% conditioned medium. It should be noted that different lots of fetal calf serum vary significantly in their ability to support clonal growth. We therefore screen fetal calf serum by determining its influence on cloning efficiency. We have found that only about 10% of the lots support good clonal growth. Cells can be cloned in either round- or flat-bottom microtiter wells (Linbro), but we have found that the round-bottom wells yield slightly higher cloning efficiencies. Wells are seeded with 10, 1, 0.3, or 0.1 cells/well plus irradiated PBLs (2000 rad, 8×10^4 cells/well) or tumor cells (10,000 rad, 2×10^4 cells/well).

Colonies are visible after 1–3 weeks and are then split into flat-bottom microtiter wells containing 5×10^5 irradiated PBLs or 2×10^4 irradiated tumor cells. Colonies are then tested for cytotoxic activity, specificity, and phenotype. After cells are expanded to 4–8 wells, they are split into 2-ml wells (Linbro) containing 10^6 PBLs or 10^5 tumor cells. Allospecific human CTLs are assessed for specificity using panels of EBV B lymphoblasts as targets of known HLA types. Specificity is further confirmed by monoclonal antibody inhibition of specific cytolysis. Aliquots of each line are frozen (10% DMSO/90% fetal calf serum v/v) and stored in liquid nitrogen. Clones are subcloned by limiting dilution to ensure clonality.

3. Results and Discussion

3.1. The Use of CTL Lines and Clones to Define Allogeneic Target Molecules

For many years it was assumed that human allogeneic CTLs recognized only the HLA-A and -B antigens.[10] We and others have recently shown that CTLs can also recognize HLA-C,[11] HLA-DR,[12–16] DC,[17,18] and SB[19] target molecules.

The HLA-A and -B molecules induce the strongest allogeneic CTL response.[20] When peripheral blood lymphocytes are stimulated by cells expressing foreign class I (HLA-A, B, C) and class II (HLA-DR, DC, and SB) MHC antigens, CTLs of a variety of target specificities are generated.[21] After 3–4 weeks in culture the dominant target antigens recognized are the HLA-A and -B antigens.[12] If, however, class I MHC disparity between responding PBLs and stimulator cells is eliminated, class II-specific CTLs can be generated and propagated in long-term culture. For example, the system may be biased by using stimulator cells that are allogeneic at only some of the MHC loci. Albrechtsen *et al.*[22] and Feighery and Stastny[23] used this protocol to generate mixed lymphocyte cultures between HLA-A, B identical donors and showed that CTLs

could recognize non-class I MHC molecules, presumably class II molecules. We used a different approach, in which peripheral blood lymphocytes were stimulated with cells that do not express certain MHC antigens. Daudi is an Epstein–Barr virus (EBV)-transformed lymphoblastoid cell line that does not express class I MHC antigens due to an inability to produce β_2-microglobulin. When peripheral blood lymphocytes were stimulated with a cell line (JY) that expresses both class I (HLA-A2, A2; B7, B7) and class II (HLA-DR4, 6; DC1, SB2, 4) antigens, the long-term CTL lines generated were specific for HLA-A2 and/or HLA-B7 determinants.[12] If, however, peripheral blood lymphocytes were stimulated with the JY cell line only once, followed by continued stimulation with the Daudi cell line (HLA-A^-, B^-, C^-, DR6; DC1; SB2), CTLs specific for HLA-DR6[12,13] or DC1[17] determinants were generated. Finally, Moretta *et al.*[24] derived largely $OKT4^+$, rather than $OKT8^+$, CTLs by cloning immediately after primary stimulation regardless of the MHC antigens expressed by the stimulator cells.[24]

Once the CTL lines have been generated, target specificity is determined by: (1) testing cytolysis of panels of EBV-transformed target cells expressing a variety of MHC antigens and (2) testing the ability of a panel of anti-MHC monoclonal antibodies to inhibit specific cytolysis.[12] Using such techniques, we and others have generated human allogeneic CTL lines and clones specific for HLA-A, B,[12,20] -C[11] and -DR,[12–16] SB,[19] and DC[17,18] MHC antigens.

At any point after primary stimulation, CTLs can be cloned by limiting dilution[9] or micromanipulation.[25] Clones of T cells arising from a single cell have been used to probe the variety of antigenic specificities recognized by T cells. Results from these studies have shown that CTLs may recognize a number of different determinants on a single molecule[26] and that T cells may recognize determinants other than those recognized serologicially, i.e., by antibodies.[27] CTL clones have also helped to define variants of MHC molecules.[28] For example, HLA-A2 was originally identified using alloantisera, and approximately 60% of North American Caucasians were found to be HLA-A2 positive.[29,30] Using CTL lines and clones as probes, however, subsets or "variants" of HLA-A2 have been demonstrated. Recent biochemical studies have identified the molecular and structural basis for these cell-mediated lympholysis-defined determinants.[31,32] In these ways, CTL lines and clones have provided important information regarding antigen recognition by lymphocytes.

3.2. Transfection of Cloned MHC Genes to Study Target Molecules

Advances in molecular biology have provided a new approach to the study of CTL specificity and recognition of target cell MHC antigens. Genes encoding various MHC antigens have been cloned and transfected into eukaryotic cells by DNA-mediated gene transfer, allowing analysis of the CTL interaction with individual gene products.[33] Murine L cells have been transfected with H-2 genes, but recently several groups have reported that human CTLs do not recognize HLA genes transfected and expressed in L cells.[34,35] When these same

genes were transfected into human cells (an osteosarcoma and a rhabdomyosarcoma) allospecific human CTL clones could lyse the transfected cells.[36] It is unlikely that conformational changes induced by coexpression with murine β_2-microglobulin accounts for the lack of recognition.[35] Possible explanations include: (1) differences in species-specific posttranslational modification, (2) recognition of MHC + X, where X is missing from the transfected cells, and (3) lack of some critical cell surface molecule essential for cytolysis (see below).

3.3. Functional Studies of Cell Surface Antigens: The Importance of the OKT4 and OKT8 T-Cell Surface Molecules

Human T cells can be classified not only by function, but also by cell surface molecules defined by monoclonal antibodies, such as the OKT[37] and Leu[38] series of antibodies. All peripheral blood T cells bear the OKT3 (Leu-4) antigen. It was originally thought that OKT4 (Leu-3) antigen was exclusively found on the helper/inducer subset of T cells and the OKT5/OKT8 (Leu-2) antigens were specific for the suppressor/cytotoxic subset.[39] However, when CTLs specific for HLA-DR6 antigens were analyzed, they were found to express OKT4 and not OKT8[12]; thus, the CTLs expressed the phenotype thought specific for the helper/inducer subset and lacked the marker thought specific for CTLs. On the other hand, CTLs specific for HLA-A2 and -B7 antigens expressed OKT8 and not OKT4, as expected.[12] These findings raised the possibility that the OKT4 and OKT8 molecules are involved in the recognition of HLA-DR (class II) and HLA-A, B (class I) molecules, respectively, rather than defining the function of T-cell subsets.[12–16] This hypothesis was supported by numerous reports of OKT4$^+$ CTLs,[12–16] OKT8$^+$ helper cells,[40] and OKT4$^+$ suppressor cells.[41]

Although the hypothesis that OKT8 may be a receptor for class I MHC antigens (recognizing a monomorphic site on the MHC molecule) and that OKT4 is a receptor for class II MHC antigens is attractive, exceptions to this correlation have already been described. We have reported a DC1-specific CTL line that expresses OKT8 and not OKT4.[17] Similarly, other OKT8$^+$ HLA-DR-,[16,42] DC-,[18] and SB[19]-specific CTLs have been described.

To test whether these cell surface molecules were functionally involved in the CTL–target cell interaction, we examined the ability of anti-T-cell monoclonal antibodies to inhibit directly the CTL–target cell interaction. OKT3 (Leu-4) monoclonal antibody inhibits cytolysis by all the CTLs that we studied. Biddison *et al.*[43] and Moretta *et al.*,[44] however, have recently described OKT3$^+$ CTLs that are not inhibited by anti-OKT3 monoclonal antibodies. OKT4 (Leu-3a) monoclonal antibodies inhibited only the HLA-DR6 (OKT4$^+$)-specific CTLs, and OKT8 (Leu-2a) monoclonal antibodies inhibited only the HLA-A2 and -B7 (OKT8$^+$) CTLs.[13,45] Of particular note, neither OKT4 nor OKT8 monoclonal antibodies inhibited cytolysis by the OKT8$^+$, DC1-specific CTLs.[17] Frequently, when the OKT8-class I/OKT4-class II dichotomy does not hold, neither OKT4 nor OKT8 monoclonal antibodies block cytolysis.[45]

The ability of anti-T-cell antibodies to inhibit CTL–target cell conjugate formation has also been examined. Landegren *et al.*[46] and Tsoukas *et al.*[47] have shown that OKT8 (Leu-2) inhibits CTL–target cell conjugation, while OKT3 does not. We recently confirmed these results and also showed that anti-OKT4 monoclonal antibodies inhibit conjugation between an OKT4$^+$ CTL and its target.[48]

Thus the precise role of the OKT4 and OKT8 T-cell surface molecules remains unclear. Although they divide peripheral blood lymphocytes into two almost mutually exclusive subpopulations (there are rare OKT4$^+$, OKT8$^+$-peripheral blood lymphocytes), OKT4 and OKT8 are not simply associated with the helper/inducer and cytotoxic/suppressor functions as once thought. Neither are they strictly associated with either class I or class II MHC recognition.

3.4. Lymphocyte Function-Associated Antigens (LFA)-1, 2, and 3

To define other function-associated cell surface molecules, we generated a panel of anti-human CTL monoclonal antibodies and screened them for their ability to inhibit T-cell functions.[49] Springer and co-workers had previously used a similar protocol to identify functionally relevant epitopes on the murine cell surface molecules Lyt-2 (the homologue of OKT8) and lymphocyte function-associated antigen-1 (LFA-1).[50] We immunized mice with an OKT4$^+$, HLA-DR6-specific CTL line, and selected hybridomas for the ability to inhibit cytolysis by the immunizing cell line. Monoclonal antibodies specific for three different molecules, designated LFA-1, 2, and 3, inhibited cytolysis, suggesting that these cell surface structures participate in the CTL–target cell interaction.

Seven of the hybridomas produced antibodies that immunoprecipitated LFA-1 polypeptide chains of 177 and 95 kD from CTLs. One monoclonal antibody (TS1/18) recognizes the β chain (95 kD) of LFA-1, while all of the other monoclonal antibodies recognize the α chain (177 kD).[51] Three unique and three partially overlapping epitopes of human LFA-1 have been identified by competitive cross-inhibition binding assays using biosynthetically labeled anti-LFA-1 monoclonal antibodies.[50] Quantitative differences in the ability of different monoclonal antibodies to block cytolysis suggest distinct functional as well as antigenic epitopes.[50]

LFA-1 is expressed on 95% of peripheral blood lymphoctyes (PBLs), including both B and T lymphocytes, as well as the majority of thymocytes, phytohemagglutinin (PHA)-activated blasts, CTLs, granulocytes, monocytes, and one-third of bone marrow cells.[52] Immunoprecipitation and FACS analyses show quantitative differences in the expression of LFA-1 on various cell types: CTLs and PHA-activated cells >PBLs >thymocytes >B lymphoblastoid cells.[52] The broad distribution of LFA-1 on lymphoid cells prompted us to assess the ability of anti-LFA-1 monoclonal antibodies to block a variety of immune responses. Anti-LFA-1 monoclonal antibodies significantly inhibit: (1) cytolysis by OKT8$^+$, HLA-A, B-specific CTLs; OKT4$^+$, HLA-DR-specific CTLs; OKT8$^+$, DC1-specific CTLs, and NK cells[52]; and (2) proliferative responses to alloan-

tigens (MLR), mitogens (PHA, Con A), and soluble antigens.[52] Complete blocking is achieved with 1–5 μg/ml of antibody.

Two other groups have recently reported monoclonal antibodies that also appear to recognize the LFA-1 molecule. Hildreth *et al.*[53] immunized mice with an EBV-transformed human lymphoblastoid cell line and generated two monoclonal antibodies, MHM23 and MHM24, which immunoprecipitate 180- and 95-kD polypeptide chains from both B and T lymphocytes. Both antibodies inhibit HLA-restricted lysis of influenza-infected and EBV-transformed target cells by binding to the effector cell.[53] Recently, Beatty *et al.*[54] described another monoclonal antibody, 60.3, which has similar properties. Further insight into the role of the LFA-1 molecule has recently been made possible by the discovery of immunodeficient patients who lack the LFA-1/Mol family of cell surface molecules (Refs. 45 and 55–60, and Springer and Anderson, this volume, Chapter 11). These patients have recurrent bacterial infections and impaired granulocyte phagocytosis. This group of patients offer a unique opportunity to study the functional role of the LFA-1 molecule.

We have studied three LFA-1-deficient patients, their families, and unrelated individuals. In all three cases, cells from the patients show decreased CTL-mediated cytotoxicity, NK-cell-mediated cytotoxicity, and proliferative responses to alloantigens and PHA.[45,56,60]

The broad cellular distribution of LFA-1 and its involvement in many immune functions suggest that, analogous to murine LFA-1, this molecule is involved in cell–cell adhesion. We therefore examined the effect of anti-LFA-1 on CTL–target cell conjugate formation. As expected, anti-LFA-1 blocked conjugate formation between CTLs and lymphoblastoid targets.[48]

The LFA-2 antigen is defined by two monoclonal antibodies that inhibit cytolysis and immunoprecipitate a diffuse band of 49 kD from thymocytes, PHA blasts, and CTLs, but not from granulocytes, monocytes, or B cells.[52] This antigen is expressed on PHA blasts >CTLs >thymocytes >PBLs, whereas OKT3 expression is similar on resting and activated T cells.[52] Further analysis showed that LFA-2 is identical to the "sheep erythrocyte receptor molecule," also recognized by OKT11,[61] Leu-5,[62] and monoclonal antibody 9.6.[63] The MAbs to this molecule inhibit the formation of spontaneous rosettes between T lymphocytes and sheep erythrocytes. This rosetting reaction has long been used to advantage by immunologists for the purification of human T lymphocytes on density gradients. All of these monoclonal antibodies inhibit cytolysis by HLA-A,B-, HLA-DR-, and DC-specific CTLs and block proliferative response to alloantigens, mitogens, and soluble antigens.[52] Only OKT11 completely modulates this molecule from the cell surface (anti-LFA-2, Leu-5, and 9.6 modulate 50–75% of the molecule from the cell surface). This indicates that complete modulation of the "sheep erythrocyte receptor molecule" is not necessary for functional inhibition.

Martin *et al.*[64] recently demonstrated that 9.6 and another monoclonal antibody, designated 35.1, recognize different epitopes in close proximity on the sheep erythrocyte receptor molecule, but that 9.6 inhibits E rosette formation and cell-mediated cytotoxicity, while 35.1 does not. Of note, these au-

thors found that 9.6 also inhibits NK cell-mediated cytolysis.[64] In our system, anti-LFA-2 monoclonal antibodies (TS2/18, TS1/8) fail to inhibit NK-cell-mediated cytolysis.[52]

Anti-LFA-2 monoclonal antibody precipitates molecules of different molecular weights from T cells at different stages of activation and/or differentiation.[52] Since LFA-2 is a glycoprotein, this heterogeneity may be due to differences in either glycosylation or amino acid sequence. Because of the apparent similarity between the molecular weight of the putative T-cell receptor and the sheep erythrocyte receptor, LFA-2 was immunoprecipitated from a series of CTL clones and found to be of uniform molecular weight. In addition, preclearing experiments showed a lack of identity between the putative T-cell receptor and LFA-2. Lastly, comodulation experiments confirmed that OKT3 and Ti reciprocally comodulate, while OKT3 and LFA-2 do not.

Thus, the "sheep erythrocyte receptor" recognized by anti-LFA-2 and a number of other monoclonal antibodies is a T-cell function-associated molecule. It has a slightly broader cell distribution than OKT3.[52] We have shown that anti-LFA-2 monoclonal antibodies inhibit CTL–target cell conjugate formation.[48] Meuer *et al.*[65] have recently suggested that this cell surface antigen is involved in an "alternative" T-lymphocyte activation pathway separate from the OKT3 circuit.[65] The precise role of this T-cell function-associated molecule remains undefined.

LFA-3 (45–65 kD) is expressed on 40–60% of peripheral blood lymphocytes, including both B and T lymphocytes. It is also present on essentially all monocytes, granulocytes, CTLs, B-lymphoblastoid cell lines, platelets, vascular endothelial cells, smooth muscle, and fibroblasts.[52] Anti-LFA-3 monoclonal antibody blocks cytolysis by CTLs, but not NK cells, and inhibits all T-cell proliferative responses studied.[52]

To establish the locus of inhibition of anti-LFA monoclonal antibodies, effector or target cells were pretreated with anti-LFA-1, anti-LFA-2, or anti-LFA-3 monoclonal antibodies, and washed extensively, and inhibition of cytolysis was assessed in a standard ^{51}Cr-release assay. Both anti-LFA-1 and anti-LFA-2 monoclonal antibodies block cytolysis by binding to the effector cell, while anti-LFA-3 monoclonal antibody blocks cytolysis by binding to the target cells.[52]

In recent studies, we have shown that trypsin treatment of target cells removes LFA-3 but leaves LFA-1 and MHC molecules intact on the lymphoblastoid target cell surface.[66] Trypsin treatment of target cells results in increased blocking by anti-LFA-1 monoclonal antibodies, but a loss of inhibition by anti-LFA-3 monoclonal antibody. These findings confirm that LFA-3 is involved in the CTL–target cell interaction at the level of the target cell. Furthermore, they indicate that a trypsin-sensitive molecule (perhaps LFA-3) on the target cell is involved in the LFA-1–target cell interaction.

LFA-3 is a novel target molecule: It is present on all tissues studied and blocks function by binding to the target cell rather than the effector cell. Of note, immunoprecipitation of LFA-3 from lymphocytes, fibroblasts, and endothelial cells indicates a molecular weight heterogeneity from 50,000 to

60,000.[52] Studies to determine if this heterogeneity is due to carbohydrate or protein differences are in progress.

3.5. The Role of Cell Surface Antigens in the T-Lymphocyte–Mesenchymal Cell Interaction

The mechanism of CTL-mediated lysis has been largely studied using lymphoid and other bone marrow-derived target cells. Although the use of lymphoid target cells is experimentally convenient and has provided great insight into the CTL–target cell interaction, the pathophysiologically relevant targets in organ transplantation include other cells, such as vascular endothelium and stromal cells. It is possible that CTL recognition and lysis of these cell types proceeds through different mechanisms, perhaps involving different accessory molecules. We have shown that an HLA-DR6-specific CTL clone, generated against lymphoblastoid targets, can specifically recognize and lyse cultured human endothelial cells and fibroblasts that have been induced to express HLA-DR antigens by immune interferon.[67] We have used this system to show that the CTL–vascular endothelium and CTL–fibroblast interactions also involve at least OKT3, OKT4/OKT8, LFA-1, LFA-2, and LFA-3 molecules.[68]

4. Summary

A number of cell surface molecules have been identified that appear to be important in the human CTL–target cell interaction (Table I). The T3–Ti complex appears to be involved in antigen-specific T-cell interactions.[69] Meuer and his colleagues[69] defined the antigen-specific receptor (Ti) on T cells in man with a clonotypic (clone-specific) monoclonal antibody that blocks lymphocyte function. The T3 molecule is coprecipitated and comodulated with Ti. Monoclonal antibodies to T3 block all T-cell functions studied and are mitogenic.[69] Until recently, all monoclonal antibody blocking experiments indicated a requirement for OKT3. Moretta *et al.*[44] and Biddison *et al.*,[43] however, have described CTL clones that appear not to require the OKT3 molecule; cytolysis by these $OKT3^+$ CTLs is not inhibited by anti-OKT3 monoclonal antibodies. Thus, no single cell surface molecule has been shown to be "required" for lymphocyte function.

In addition to the antigen-specific receptor, other "accessory" (non-antigen-specific) molecules appear to be involved in the CTL-target cell interaction. These molecules appear to function, at least in part, by increasing the overall CTL–target cell affinity. High-affinity interaction between the T-cell receptor and antigen may be sufficient to allow cytolysis; alternately, low-affinity antigen–receptor interaction may require additional "accessory" molecules for cytolysis.

Martz *et al.* divided CTL-mediated cytolysis into two steps based on differing dependence on divalent cations.[70] The first step, requiring Mg^{+2} and

Table I. Human Cell Surface Molecules Associated with Lymphocyte Function

Molecule	Molecular weight	Cell distribution	Functions inhibited by monoclonal antibody		Monoclonal antibody inhibits by binding to	Proposed molecular function
			Cytotoxicity	Proliferation		
1. HLA-A, B, C	44,000, 12,000	All cells except red blood cells	+ (HLA-A, B, C-specific CTLs)	±[a]	Target	Class I MHC antigens
2. HLA-DR	34,000, 29,000	Macrophages, B cells, activated T cells, other "immune cells"	+ (HLA-DR-specific CTLs)	+	Target	Class II MHC antigens
3. SB (DP)	34,000, 29,000	Same as HLA-DR	+ (SB-specific CTLs)	+	Target	Class II MHC antigens
4. DC (DQ)	34,000, 29,000	Same as HLA-DR	+ (DC-specific CTLs)	+	Target	Class II MHC antigens
5. LFA-3	45,000, 65,000	Broad	+	+	Target	Target accessory molecule (? ligand)
6. Ti	49,000, 43,000	T Cells	+	+	Effector	Antigen receptor
7. OKT3 (Leu-4)	19,000	T Cells	+	+	Effector	Associated with Ti
8. OKT_4 (Leu-3a)	62,000	Sixty percent of T cells	+	+	Effector	HLA-DR-associated accessory molecule
9. OKT8 (Leu-2a)	43,000	Forty percent of T cells	+	+[a]	Effector	HLA-A, B, C-associated accessory molecule
10. LFA-1	177,000, 95,000	Leukocytes	+	+	Effector	Leukocyte accessory molecule (? adhesion)
11. LFA-2 (Leu-5, OKT11, E-rosette receptor)	49,000	T Cells	+	+	Effector	T-Cell accessory molecule

[a] Small subpopulation.

specific antigen recognition, involves adherence of the CTLs to the target. The studies that we have described suggest that OKT4 and OKT8 may be involved in recognition of monomorphic MHC determinants at this step. LFA-1, LFA-2, and LFA-3 similarly inhibit conjugate formation[48] and may be involved in some non-antigen-specific recognition process and/or adhesion. Once low-affinity, non-antigen-specific bonds form, the antigen-specific receptor may engage antigen. Then, depending on the avidity of the specific antigen–receptor interaction, other "stabilizing" interactions involving OKT4/OKT8, LFA-1; LFA-2, and/or LFA-3 may be required. A "lethal hit" is delivered in the second, Ca^{2+}-dependent step. Once the lethal hit occurs, the target cell is "programmed for lysis"; that is, lysis occurs even if the CTL–target conjugate is disrupted.[70] It is remarkable that the experimental methods described in this chapter have given rise only to monoclonal antibodies that recognize molecules that appear to be involved in the first step of the CTL–target cell interaction. It is possible that cell surface molecules are not involved after "triggering" of the cell, i.e., in delivery of the lethal hit.

5. Conclusion

Hybridoma technology and T-cell cloning have advanced our understanding of the molecules involved in the CTL–target cell interaction. Monoclonal antibodies selected for their ability to inhibit lymphocyte function have allowed us to define further the role of the OKT4 and OKT8 molecules and to define new lymphocyte function-associated antigens LFA-1, LFA-2, and LFA-3. These studies have demonstrated a series of accessory (not antigen-specific) molecular interactions which may be critical in the induction and stabilization of the CTL–target cell interaction. Such "accessory" molecules may be central to all cellular interactions.

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