JOURNAL OF CELLULAR PHYSIOLOGY 137:173–178 (1988)

Intercellular Adhesion Molecule-1 (ICAM-1) Is Involved in the Cytolytic T Lymphocyte Interaction With a Human Synovial Cell Line

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The cell surface molecules involved in the human cytolytic T lymphocyte (CTL)-synovial cell interaction may play an important role in T cell interactions with connective tissue mesenchymal cells. To examine the molecular basis for the CTL-synovial cell interaction, we immortalized synovial cell explants to establish the cell line SYN.SPP. The SYN.SPP cell line was compared to the established B lymphoblastoid cell line JY. Cell surface immunofluorescence demonstrated significantly different levels of the immunologically relevant cell surface molecules ICAM-1 and LFA-3. Both cell lines were used to stimulate CTL precursors. After several months in culture, CTL lines stimulated by the SYN.SPP and JY cell lines demonstrated HLA class I-directed cytolytic activity. The cell surface molecules utilized by the anti-SYN.SPP and anti-JY CTL lines were identified by monoclonal antibody (MAb) inhibition. MAb recognizing the CTL cell surface molecules CD3, CD8 and LFA-1 (CD11a) significantly inhibited CTL-mediated lysis of both target cells. An interesting observation was that the anti-SYN.SPP CTL line appeared to utilize the ICAM-1 and not the LFA-3 target cell molecule. In contrast, the anti-JY CTL line utilized the LFA-3 and not the ICAM-1 membrane molecule. These results indicate that CTL interactions with connective tissue mesenchymal cells may be regulated by a unique pattern of antigen nonspecific cell-cell interaction molecules.

The majority of the cells in the synovial membrane are intimal synovial cells (Castor, 1960; Smith, 1971; Wynn-Roberts and Anderson, 1978). These cells function to maintain the structural integrity of the joint connective tissue. Intimal synovial cells are non-phagocytic, express low levels of HLA class II molecules and do not appear to interact with the immune system in normal circumstances (Barland, et al., 1962, 1971; Smith, 1971; Schumacher and Kitridou, 1972; Wynn-Roberts and Anderson, 1978; Amento et al., 1985; Oppenheimer-Marks and Ziff, 1986; Teyton et al., 1987). In addition to synovial cells, the normal synovial membrane consists of only a few macrophages, granulocytes, and wandering lymphoid cells. The synovial fluid is also largely devoid of inflammatory cells. In some inflammatory diseases, however, there is a marked influx of lymphocytes and phagocytes into the synovial membrane and joint fluid. The effects of the cellular infiltration are most evident in rheumatoid arthritis (Harris, 1984; Lydyart et al., 1982).

Synovial cells are of immunologic interest not only for their potential role in rheumatoid arthritis, but because they provide an opportunity to study T cell recognition of a connective tissue mesenchymal cell. Previous work in T cell recognition and cell-cell interaction molecules have focused on T cell interactions with lymphocyte-derived target cells or non-lymphoid tumor cells. The molecular basis of the human CTL-synovial cell interaction may involve different molecules than those previously described.

To examine the molecular mechanisms involved in the human CTL-synovial cell interaction, we immortalized synovial cell explants using an amphotropic murine retrovirus. The cell line SYN.SPP and MAb inhibition were used in conjunction with CTL lines to investigate the antigen nonspecific cell surface molecules involved in the CTL-synovial cell interaction.

MATERIALS AND METHODS

Culture medium

Cells were grown in culture medium consisting of RPMI 1640 (M.A. Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated fetal calf serum (Gibco Laboratories, Grand Island, NY), penicillin 100

Received January 22, 1988; accepted May 11, 1988.

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Abbreviations used: CTL, cytolytic T lymphocyte; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; LFA, lymphocyte function-associated antigen; MAb, monoclonal antibodies; MFI, mean fluorescence intensity.

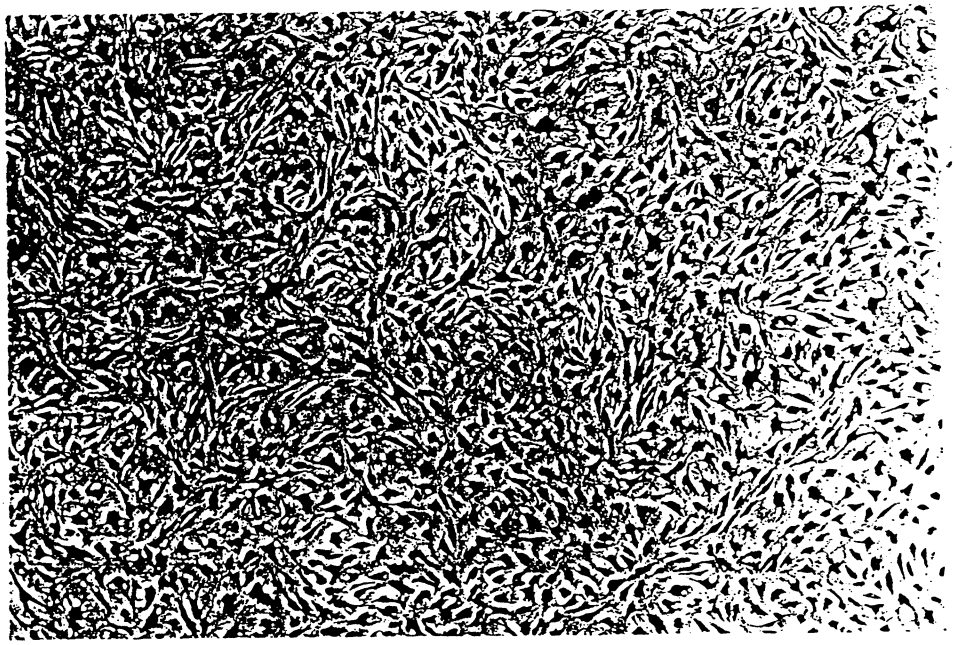

Fig. 1. Photomicrograph of the synovial cell line SYN.SPP (× 250). The immortalized synovial cells were subcultured to obtain the homogeneous cell line SYN.SPP.

μ/ml (Gibco), streptomycin 100 μ/ml (Gibco), Hepes 10 mM (Sigma Chemical Co., St. Louis, MO), and 2-mercaptoethanol 25 μM (Eastman Organic Chemicals, Rochester, NY). Long-term CTL cultures and CTL lines were maintained in culture medium and 10% conditioned medium.

Interleukin-2 containing human conditioned medium

Interleukin-2 (IL-2) containing medium was made as previously described (Krensky et al., 1985b). Briefly, peripheral blood leukocytes obtained from five different platelet donors were mixed and washed three times. The cells were resuspended at a concentration of 3×10^6 cells/ml in RPMI 1640 (M.A. Bioproducts) containing 3% fetal calf serum and 0.15% phytohemagglutinin (Bacto PHA-P, Difco, Detroit, MI). Special additives included 2.8 μM indomethacin (Sigma), 3 mM lithium chloride (Sigma), and 50 μM hydroxyurea (Sigma). The cells were cultured for 72 h and the supernatants were collected. The supernatants were ammonium sulfate precipitated (50%,75%). After dialysis, the purified conditioned medium was filter-sterilized and stored at −20°C.

CTL lines

CTL lines were established from a single volunteer donor (HLA-A11-Aw32,B27,Cw2,DR7). Peripheral blood mononuclear cells were separated on a Ficoll/Hypaque gradient (Lymphocyte Separation Medium, Bionetics Laboratory Products, Charleston, SC). The mononuclear cells at 2×10^6 cells/ml were co-cultured with irradiated JY cells (1×10^5 cells/ml; 5,000rads) or SYN.SPP (1×10^4 cell/ml;2,500 rads) in 2 ml wells (Linbro Chemical Co., Hamden, CT) at 37°C in a 5% CO_2 incubator. The bulk culture was maintained by stimulation every 1–2 weeks with irradiated stimulator cells. After 4 weeks in culture, medium containing 10% human conditioned medium was used for cloning; fresh medium was added every 3 days.

Monoclonal antibodies

Monoclonal antibody producing hybridoma cells were grown in BALB/c mouse ascities. The specificities of the HLA MAb W6/32 (monomorphic HLA-A,B,C) and LB3.1 (monomorphic HLA-DR) have been previously described (Colombani et al., 1982). MAb recognizing the lymphocyte function-associated antigens were the same as those described elsewhere (Sanchez-Madrid et al., 1982): LFA-1 (CD11a) (TS1/18, TS1/22), LFA-2 (TS2/18), LFA-3 (TS2/9). The MAb recognizing the intercellular adhesion molecule ICAM-1, (RR1/1), has also been described (Dustin et al., 1986). Commercially available MAb were also used: OKT3 and OKT8 (Ortho Pharmaceuticals, Raritan, NJ).

Cytotoxicity assay

CTL assays were performed in triplicate in V-bottom microtiter wells (Linbro, McLean, VA). Effector cells were added in three-fold dilutions. When used as target cells, the adherent cell line SYN.SPP was harvested using EDTA/trypsin (Versene; Gibco) and washed in culture medium. The target cells, pre-incubated with 0.1 mCi of In-111 (Amersham, Arlington Heights, IL) for 2 h and washed three times, were added at 10^3 cells/well. Microtiter plates were centrifuged and incubated at 37°C for 4–8 h. After incubation, the plates were again centrifuged and the supernatants assayed for In-111 release. Specific cytotoxicity was calculated as percent cytotoxicity = 100× (cpm experimental release − cpm spontaneous release)/(total cpm − cpm spontaneous release). When blocking monoclonal antibodies were used, they were added at the start of the assay. Inhibition of cyto-

toxicity was expressed as a percent reduction of the specific cytotoxicity. The standard deviation of the triplicate wells rarely exceeded 2–4% of the specific lysis.

Immortalized synovial cell lines

Human synovial cells were obtained from a patient undergoing total joint replacement, prepared using a modification of previously described techniques (Fraser and McCall, 1965; Clarris et al., 1981), and established as early primary cultures. The cell lines were rendered immortalized by infection with a Kirsten sarcoma virus or a Moloney sarcoma virus pseudotyped with an amphotropic murine leukemia virus (Faller et al., 1988). All immortalized cell lines were morphologically and phenotypically unchanged after viral treatment. Synovial cell lines, similar to previous work with endothelial cells (Mentzer et al. 1987; Faller et al., 1988), were not transformed by any criteria, but continued to proliferate in culture without exogenous growth factors. Similar to findings with primary explants, collagenase activity was undetectable after prolonged in vitro passage.

Human tumor lines

The human tumor cell lines were maintained in culture medium and regularly passaged. All lines were typed by the HLA Tissue-typing Laboratory, Dana-Farber Cancer Institute: JY (HLA-A2,B7,DR4,DR6), Daudi (HLA–,–,DR6), HOM-2 (HLA-A3,B27,Bw4,DR1), PGF (HLA-A3,B7,DR2), LB (HLA-A28,B40,DR6).

Cell surface immunofluorescence

Cells were washed twice with phosphate buffered saline containing 2.5% fetal calf serum and 0.02% sodium azide. Approximately 10^6 cells were incubated on ice for 30 min with an excess concentration of monoclonal antibody. The cells were washed twice and stained with fluorescein-conjugated goat F(ab')2-anti-mouse IgG antibody (Tago, Burlingame, CA) diluted 1:10. The cells were incubated on ice for another 30 min. After 3 washes, the cells were fixed in 1% paraformaldehyde and analyzed on a FACS I analyzer (Beckton Dickinson & Co., Mountain View, CA).

RESULTS

Human synovial cell line

To study the cell surface molecules potentially involved in CTL reactivity with human synovial cells, we established long-term synovial cell lines. Synovial cells were obtained from a patient undergoing joint replacement surgery and immortalized using an amphotropic murine retrovirus. The cells retained the light microscopic appearance of a primary synovial cell explant (Fig. 1). The cells proliferated in culture in the presence of 10% fetal calf serum for more than 6 months. The original culture was repeatedly subcultured to obtain the homogeneous cell line SYN.SPP.

The surface phenotype of this synovial cell line was investigated using a panel of MAb. Cell surface immunofluorescence of the line SYN.SPP was compared to the B lymphoblastoid cell line JY. JY expresses high levels of HLA and other immunologically relevant target cell surface structures (Krensky et al., 1983, 1985b). MAb recognizing the non-polymorphic regions of HLA demonstrated levels of synovial cell HLA class I expression that was 68% of the level of class I expressed on JY (Table 1). The levels of HLA class I expression on the synovial cell line increased two-fold after IFN-gamma induction (data not shown). Similar to previous findings using cultured synovial cells (Amento et al., 1985), HLA class II molecules were only marginally expressed.

TABLE 1. Surface phenotype of human target cell lines[1]

MAb	Antigen	MFI SYN.SPP	MFI JY
W6/32	HLA class I	88	128
LB3.1	HLA class II	5	156
TS2/9	LFA-3	53	116
RR1/1	ICAM-1	91	40

[1]Indirect immunofluorescence analysis of the synovial cell line SYN.SPP and the B lymphoblastoid cell line JY was performed on a FACS I flow cytometer. MFI, mean fluorescence intensity was the mean relative fluorescence as assessed using a 3 log linear scale and a Consort 30 computer. The MFI of the blank fluorescence (fluorescent antibody alone) was subtracted from the specific MFI for each sample.

Two other cell surface structures, LFA-3 and ICAM-1, have been implicated in immune reactivity. LFA-3 appears to be the target cell ligand for the CTL molecule CD2 (Takai et al., 1987). ICAM-1 is another target cell surface molecule implicated in cell-cell adhesion (Dustin et al., 1986; Rothlein et al., 1986), but anti-ICAM-1 MAb have not been shown to inhibit CTL reactivity. An interesting finding was the comparison of LFA-3 and ICAM-1 expression on the two cell lines. Synovial cells expressed more than twice the level of ICAM-1 as the lymphoid target cell line JY. Conversely, the lymphoid target cell line expressed more than twice the level of LFA-3 as the synovial target cell line (Table 1).

Anti-synovial cell CTL lines

To study the interaction between CTL and synovial target cells, CTL lines were generated against the synovial cell line SYN.SPP as well as the B lymphoblastoid cell line JY. Because the size and adherence properties of the SYN.SPP line precluded detailed HLA typing, several donors were screened to ensure an adequate proliferative and cytolytic response. Peripheral blood mononuclear cells were stimulated at 1–2 week intervals with irradiated stimulator cells. CTL lines established from a typical donor ("M") are described here. The responder cells were stimulated with either SYN.SPP to established the MSY CTL lines or JY to establish the MJY CTL line. After 3 months in culture, the cells lines were tested for cytolytic activity against a panel of HLA-typed target cells. The MSY line had slightly decreased lytic activity against all targets corresponding to a relatively lower percentage of $CD8^+$cells (not shown). With an adjustment in $CD8^+$ effector concentration (by increasing the effector to target ratio), the CTL lines demonstrated comparable lysis of the cell lines expressing HLA class I antigens (Table 2).

MAb inhibition of the anti-synovial CTL line

To study the cell-cell interaction molecules utilized in the CTL-synovial and CTL-lymphoid target cell interaction, we compared MAb inhibition of the MSY and MJY CTL lines. Anti-LFA-1 MAb effectively blocked cytolytic activity by both the MSY and MJY CTL lines (Table 3). An unexpected finding was that anti-CD2 and anti-LFA-

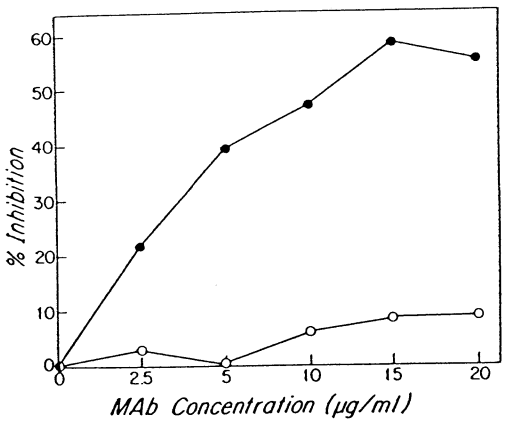

Fig. 2. Titration of the anti-ICAM-1 MAb inhibition. The MSY CTL line was tested against the SYN.SPP target cell line at an E:T ratio of 15:1 (●) and the level of SYN.SPP lysis was 68% specific release. In parallel, the MJY CTL line was tested against the JY cell line at an E:T ratio of 6:1 (○). The level of JY lysis was 74% spontaneous release. The anti-ICAM-1 MAb RR1/1 was added at varying concentrations. The effect of the RR1/1 MAb is shown as the percent inhibition of the specific In-111 release.

TABLE 2. Specificity and lytic activity of CTL lines[1]

CTL line	HLA-typed target cells				
	JY	PFG	Daudi	LB	Hom-2
MSY	80	76	23	75	37
MJY	76	71	54	64	42

[1]The anti-SYN.SPP CTL line MSY and the anti-JY CTL line MJY were tested against a panel of In-111 labeled target cells in a standard 6 h In-111 release assay at 37°C. The effector to target ratios were 15:1 for MSY and 6:1 for MJY. HLA types of the lymphoid target cells are noted in "Materials and Methods."

TABLE 3. MAb Inhibition of CTL-mediated lysis of SYN.SPP and JY target cells[1]

MAb	Antigen	% Inhibition	
		MSY	MJY
OKT3	CD3	43	51
OKT8	CD8	39	36
W6/32	HLA-A,B,C	45	34
LB3.1	HLA-DR	4	18
TS1/18	LFA-1	48	54
TS2/18	LFA-2/CD2	13	47
TS2/9	LFA-3	19	60
RR1/1	ICAM-1	57	5

[1]MAb inhibition is expressed as a percent reduction in specific In-111 release. The MSY line was tested against the SYN.SPP target cell line (E:T, 15:1), and the MJY cell line was tested against the JY cell line (E:T, 6:1). The percent specific In-111 release was 59% for the MSY CTL line and 72% for the MJY CTL line. Immunofluorescence staining of the cell lines MSY and MJY were negative for CD11b and CD11c indicating that TS1/18 was specific for CD11a/LFA-1.

3 MAb did not significantly inhibit killing by the MSY CTL line whereas both did inhibit MJY-mediated killing. This finding suggested that the MSY CTL line was not as dependent on the CD2/LFA-3 interaction as the MJY CTL line. One possible explanation for the diminished utilization of CD2/LFA-3 in target recognition by the MSY CTL line was a relatively low surface expression of LFA-3 on the SYN.SPP synovial cell line. In contrast to LFA-3, the target cell molecule ICAM-1 is expressed at relatively high levels on the human synovial cell line SYN.SPP (Table 1). To explore the potential participation of ICAM-1 in CTL-synovial cell interaction, the anti-ICAM-1 MAb RR1/1 was used to inhibit CTL-mediated lysis. The RR1/1 MAb significantly inhibited the MSY CTL-mediated cytolysis. The inhibition of the MSY CTL line by the RR1/1 MAb was obtained at IgG concentrations of 5 μg/ml. The MJY CTL line cytotoxicity, however, was not inhibited even by concentrations of RR1/1 MAb greater than 100 μg/ml (Fig. 2). These results suggested that the MSY CTL line utilized the LFA-1 and ICAM-1 cell surface structures, whereas the MJY CTL line utilized LFA-1 and CD2/LFA-3. MAb to other function-associated cell surface molecules also inhibited killing as expected (Table 3). MAb recognizing CD3 (OKT3), CD8 (OKT8), and monomorphic HLA (W6/32) comparably inhibited both MSY- and MJY-mediated cytolysis (Table 3).

Participation of ICAM-1 in the CTL-target cell interaction

The ICAM-1 cell surface molecule was previously described as an adhesion molecule in lymphocyte-fibroblast interactions (Dustin et al., 1986; Rothlein et al., 1986; Marlin and Springer, 1987). The ICAM-1 molecule is expressed on epithelial cells, fibroblasts and some hematopoietic cells, but is only marginally expressed on CTL clones. To confirm that ICAM-1 inhibited killing at the level of the target cell, we performed MAb pretreatment of the CTL line MSY and the synovial target cell line SYN.SPP (Table 4). Maximal inhibition was obtained with pretreatment of the SYN.SPP target cell. Pretreatment of both MSY and SYN.SPP or the addition of soluble MAb did not increase the level of inhibition. Thus, anti ICAM-1 MAb, similar to anti-LFA-3 MAb (Krensky et al., 1983), appeared to inhibit killing at the level of the target cell.

Selective involvement of the ICAM-1 cell surface molecule

The novel observation that ICAM-1 was involved in CTL-synovial cell interaction suggested that the utilization of ICAM-1 depended on increased expression of ICAM-1. Enhanced expression of ICAM-1 may be important on the target cell and/or the stimulator cell. To discriminate these possibilities, we tested the MSY and MJY CTL lines against both the SYN.SPP and JY target cells. The anti-ICAM-1 MAb did not inhibit the killing of the JY cell line (Table 5); that is, neither the MSY or the MJY CTL line was significantly inhibited by the RR1/1 MAb when the JY cell line was used as the target cell. In addition, the RR1/1 MAb did not inhibit MJY-mediated lysis of the SYN.SPP target cell. In multiple experiments, anti-ICAM-1 MAb inhibition was restricted to the MSY-SYN.SPP cytolytic interaction (Table 5). These results suggest that ICAM-1 participation in the CTL-target cell interaction requires both a target cell expressing a high level of ICAM-1 as well as the expansion of CTL precursors that utilize the ICAM-1 molecule.

TABLE 4. Pretreatment of CTL and target cells with anti-ICAM-1 MAb[1]

MAb treatment	Percent	
	Specific lysis	Inhibition
None	53	—
CTL	36	32
TC	19	64
CTL + TC	19	64
MAb added	19	64

[1]MAb treatment involved a 20 min pretreatment of the CTL or target cell (TC) at room temperature. The anti-ICAM-1 MAb (RR1/1) was used at greater than five-fold saturating concentrations. After incubation, the cells were washed 3 times and added to the appropriate wells. Pretreatment was compared to cells pretreated with control media (no MAb) and the addition of an identical concentration of MAb prior to the assay (MAb added). The cytolytic assay was a standard 6 h In-111 release assay at 37°C. The MSY CTL line was used at an E:T ratio of 15:1.

TABLE 5. Selective inhibition of MSY CTL and SYN.SPP target cells by the anti-ICAM-1 MAb[1]

CTL line	JY		SYN.SPP	
	No MAb	RR1/1	No MAb	RR1/1
MSY	53	45(16)	60	24(61)
MJY	75	70(7)	63	55(12)

[1]The cytolytic activity of the MSY (E:T, 15:1) and MJY (E:T, 6:1) CTL lines were assessed against the JY and SYN.SPP cell lines in a 6 h In-111 release assay at 37°C. The anti-ICAM-1 MAb (RR1/1) was used at a five-fold saturating concentration.

DISCUSSION

Primary synovial explants generally do not undergo prolonged cell division and survive only a few weeks in culture (Fraser and McCall, 1965; Smith, 1971) making long-term immunologic studies difficult. To obtain a stable synovial cell line to use as both stimulators and target cells for human CTL, we used an amphotrophic murine retrovirus to immortalize human synovial cells. Similar to earlier work using endothelial cells and fibroblasts (Mentzer et al., 1987; Faller et al., 1988), viral infection resulted in synovial cell proliferation without morphologic transformation. The SYN.SPP cell line used in these studies was morphologically and antigenically stable for more than 6 months in culture.

The synovial cell line SYN.SPP was initially used to stimulate the generation of CTL precursors. In comparison with lymphoid cells, synovial cells were much less efficient in stimulating CTL precursors to develop into mature antigen specific CTL. Peripheral blood donors representing multiple HLA types were screened to obtain a lymphocyte population with a proliferative and cytolytic response that would permit long-term study. Because of the variety of HLA types tested, the relative inefficiency of the SYN.SPP cell line as stimulator cells did not appear to be the result of HLA polymorphism but more likely was the result of a relative deficiency of antigen nonspecific cell-cell interaction molecules expressed on the SYN.SPP cell surface. Consistent with this possibility, we found that the SYN.SPP cell line expressed undetectable levels of LFA-1 (CD11a) as well as low levels of other immunologically relevant cell surface molecules such as HLA class II and LFA-3. All 3 of these molecules have been functionally implicated in the generation of mature CTL (Engleman et al., 1981; Krensky et al., 1983; Krensky et al., 1985a).

The synovial cell line SYN.SPP did, however, function as target cells for CTL-mediated lysis. The relative level of anti-SYN.SPP lytic activity was correlated with the expression of HLA class I molecules. The SYN.SPP cell line expressed 68% of the level of HLA class I molecules expressed on the JY cell line. However, the SYN.SPP cells were approximately three-fold larger than JY cells by flow cytometry (data not shown). Thus, the relative surface density of HLA class I molecules was less than 23% of the density on JY. Although the density of HLA class I expression was much less than JY, it was comparable to the surface density of other large mesenchymal cell lines (Mentzer et al., 1986). Despite the difference in the surface density of HLA class I molecules on the SYN.SPP and JY cell lines, both the MSY and MJY CTL lines appeared to recognize the HLA class I target molecule. The HLA-typed target cell panel as well as anti-HLA-A,B,C, MAb blocking indicated that the dominant target molecule for both of these allospecific CTL lines was HLA-A2.

MAb inhibition of the CTL and target cell lines demonstrated an important difference in the utilization of the LFA-3 and ICAM-1 target cell molecules. Both LFA-3 and ICAM-1 are membrane molecules that appear to function as antigen independent cell-cell interaction molecules. LFA-3 is a 60 kd glycoprotein expressed on virtually all human cells (Sanchez-Madrid et al., 1982; Krensky et al., 1983). Previously, the LFA-3 membrane molecule was the only non-HLA target cell molecule implicated in CTL reactivity. An explanation for the involvement of LFA-3 in CTL reactivity is the recent evidence that LFA-3 is the cell surface ligand for CD2. The CD2/LFA-3 receptor-ligand interaction may be important for cell-cell adhesion as well as triggering CTL activation.

ICAM-1 is a 97kd cell surface molecule that also demonstrates some tissue heterogeneity in molecular weight (Dustin et al., 1986). The ICAM-1 molecule is expressed in a variety of mesenchymal cells including fibroblasts and synovial cells (Dustin et al., 1986; Rothlein et al., 1986). In contrast to LFA-3, ICAM-1 has not been previously implicated in CTL-mediated killing. The potential role for ICAM-1 in cell-cell interactions has been suggested by the ability of anti-ICAM-1 MAb to inhibit antigen independent adhesion of lymphoid cells to fibroblasts (Dustin et al., 1986; Rothkein et al., 1986). Purified ICAM-1 protein has recently been shown to specifically bind to the LFA-1 membrane molecule (Marlin and Springer, 1987) indicating that the ICAM-1 molecule is a physiologic receptor for the LFA-1 molecule. Using the SYN.SPP cell line and MAb inhibition, we have shown that ICAM-1 participates in CTL-mediated lysis at the level of the target cell. This finding suggests that the ICAM-1 molecule can function in a receptor/ligand interaction with the LFA-1 molecule.

An interesting observation was the inability of anti-ICAM-1 MAb to significantly inhibit killing of an ICAM-1^+ lymphoid target cell. The inhibition of CTL by anti-LFA-1 MAb, in the absence of anti-ICAM-1 MAb blocking, suggests that LFA-1 may utilize other, as yet unidentified, cell surface ligands.

The cell surface molecules expressed on the synovial cell line SYN.SPP appeared to be an important deter-

minant of the lymphocyte accessory molecules utilized in the CTL-target cell interaction. CTL precursors that were initially stimulated with synovial cells utilized the ICAM-1 molecule in the CTL-synovial cell interaction. In contrast, when CTL precursors from the same donor were initially stimulated with lymphoid cells, they did not utilize the ICAM-1 cell surface molecule in their subsequent target cell interactions. MAb inhibition experiments suggested that these latter CTL appeared to be more dependent on the CD2/LFA-3 interaction that the ICAM-1-ligand interaction. Our observations suggest that stimulation of CTL precursors by cells of different phenotype or tissue origin can result in the amplification of CTL that can utilize different cell-cell interaction molecules. Thus, the specificity of the cell-mediated immune response appears to be controlled not only by the T cell receptor, but by antigen nonspecific cell-cell interaction molecules as well. The utilization of a particular pattern of cell-cell interaction molecules may be required for effective T cell interaction with different tissues. In this study, the use of the immortalized allogeneic cell line SYN.SPP permitted the study of the molecular mechanisms involved in the CTL-synovial cell interaction. Two different target cell ligands, LFA-3 and ICAM-1, appear to play distinct and significant roles in stimulating the generation of mature CTL. These molecules also participate in CTL-mediated target cell destruction. The function of the ICAM-1 molecule was restricted to the CTL-SYN.SPP interaction. Thus, in this system, the pattern of accessory molecule expression on the surface of the stimulator cell regulated the participation of the CTL cell surface molecules utilized in the subsequent lytic interactions. These experiments, using an intimal synovial cell line, suggest that the differential utilization of cell-cell interaction molecules may also apply to T cell interactions with fibroblasts and other mesenchymal cells. Future studies will attempt to elucidate the role of these CTL-synovial cell interaction molecules in the normal and pathogenic immune response.

ACKNOWLEDGMENT

This study was supported by NIH grants 09280, 36179, and 34128.

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