to functionally different complexes. Further studies using fragments of both the CD2 and CD16 antibodies will be required to understand the mechanism of activation, but it would appear that both molecules will play a significant role in NK activation.

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T2.9 Human cytotoxic T-cells adhere to potential targets by two antigen-independent pathways: CD2 binding to LFA-3 or LFA-1 binding to an undefined ligand

S. SHAW, G.G. LUCE, T.A. SPRINGER, M.L. PLUNKETT, R. QUINONES, R.E. GRESS, and M.E. SANDERS

T-cell mediated cytotoxicity proceeds in three distinct steps: (1) formation of a stable adhesion between effector and target (conjugate formation); (2) effector delivery of a 'lethal hit' which initiates damage to the target; and (3) target cell lysis [1]. Studies from murine model systems have indicated that conjugates are formed principally with targets which express the relevant antigen [2]. In contrast, our studies demonstrate marked antigen-independent conjugate formation by human cytotoxic T-cells. Monoclonal antibody (mAb) inhibition studies with individual mAbs and mixtures of mAbs demonstrate that LFA-1 (CD18) is involved in one pathway of adhesion, which is functional at 37°C but not at 4°C and requires Mg^{2+}. Effector CD2 (T11, LFA-2, E-rosette receptor) binding to target LFA-3 is a distinct adhesion pathway which functions at 4°C as well as at 37°C and is independent of divalent cations. Although both pathways function in conjugate formation with a variety of *in vitro* cell lines, they can apparently function independently. The CD2/LFA-3 pathway mediates rosetting of T-cells with human erythrocytes. The LFA-1 pathway is the primary mode of interaction of T-cells with monocytes.

The model system in which we have studied conjugate formation most extensively is in the interaction of human cytotoxic T-lymphocyte (CTL) clones with *in vitro* cell lines. The clones lyse only targets which express the sensitizing alloantigen (DPw2) [3; Sanchez-Perez, Petersen, DeMars, and Shaw, submitted for publication]; nevertheless, the clones form conjugates not only with target cells that express DPw2 but also with those which do not. A variety of approaches have failed to distinguish the conjugates formed with antigen-bearing targets from those with antigen-negative targets (e.g. strength or kinetics of formation). Because of the contrast with findings in the murine system, several factors which might contribute to conjugate formation have been analysed and discounted (e.g. lectin-bridging, peculiarities of long-term T-cell culture, abnormalities of transformed targets). On the contrary, several lines of evidence convince us that the observed antigen-independent conjugate formation is physiologic and that initial conjugate formation may be principally mediated by antigen-independent mechanisms: (1) antigen-independent conjugate formation (of varying magnitude) has been observed in many different systems; (2) the mAb which we find inhibit antigen-independent conjugate formation (αLFA-1, CD2, αLFA-3, see below) are ones already known to inhibit the overall cytotoxic interaction [4]; (3) theoretical considerations on cell adhesion indicate that major repulsive forces must be overcome [5] and suggest to us that antigen-independent adhesion may

Table 1. Additive inhibition of conjugate formation by mixes of αLFA-1 with either CD2 or αLFA-3 mAb

	Per cent conjugates in presence of first antibody			
Second antibody	None	LFA-1	CD2	LFA-3
None	70	51	44	40
LFA-1	51		0	0
CD2	44	0		29
LFA-3	40	0	29	

Conjugate formation between clone 8.2 and target U266 at a 4:1 E:T ratio following pelleting and incubation for 6 min at 37°C. Conjugates are enumerated by dual fluorescence flow cytometry and expressed as the per cent of targets which become attached to an effector [9]. Inhibitory antibodies are purified IgG at 100 μg/ml which are present continuously during conjugate formation; inhibition is not increased at higher concentrations of antibody. Note the axis of symmetry of Table 1 and that values are repeated to facilitate visual comparisons.

provide optimal alignment of membranes for long enough to allow the T-cell's antigen receptors to encounter specific antigen, even when present at low concentration on the target.

We find that mAbs against LFA-1, CD2, and LFA-3 each *partially* inhibit antigen-independent (as well as antigen-specific) conjugate formation ([6] and Table 1). Studies of mixes of mAbs (Table 1) demonstrate that mixes of αLFA-1 with either CD2 or αLFA-3 mAb consistently abrogate conjugate formation; however, there is at most a modest additive effect of CD2 plus αLFA-3 mAb. These findings with purified IgG (previously seen with ascites preparations of mAbs [6] and recently with Fab preparations of mAbs, not shown) suggest that CD2 and LFA-3 molecules may function in one pathway of conjugate formation and LFA-1 in another. This hypothesis is supported by studies showing that there are differences between the pathways in their inhibition by reduced temperature and by chelation of divalent cations ([6] and Table 2). The LFA-1 pathway (which remains after inhibition with either CD2 or αLFA-3 mAb) is inhibited by reducing temperature to 4°C during cell contact or by chelation of divalent cations. In contrast, neither temperature reduction nor cation chelation affect the CD2/LFA-3 pathway (which remains after inhibition with αLFA-1). In our studies it is evident that the CD2 molecule functions on the effector cell since it is generally not expressed on the targets. Although the LFA-1 and LFA-3 molecules are expressed both on the effector and target, studies with mAb washout following pre-incubation of either effector or target [Shaw, Luce, Quinones, Gress, Springer, and Sanders, in preparation], as well as

Table 2. Two pathways of conjugate formation differ in temperature sensitivity and divalent cation requirements

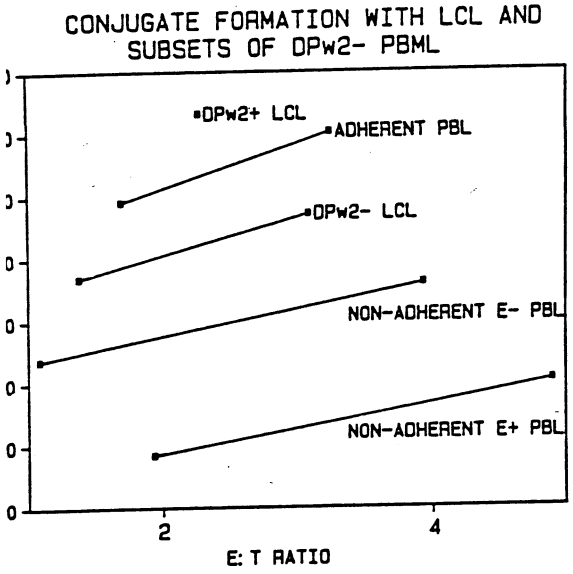
			Per cent conjugate formation for antibodies			
Temperature (°C)	Time (min)	EDTA	None	αLFA-1	αCD2	αLFA-3
37	6	No	49	22	34	33
4	25	No	21	28	0	0
37	6	7.5 mM	27	23	0	0

Conjugate formation between clone 8.9 and target U266. Experimental details are as in Table 1 except for differences noted in Table 2 in temperature of incubation during conjugate formation, duration of that incubation, and presence of chelating agent.

Table 3. Characteristics of two pathways of antigen-independent conjugate formation by human T-cell clones

Pathway	Receptor on T-cell	Ligand on target	Divalent cation requirement	Inhibition at 4°C
1	LFA-1	?	Mg^{2+}	Yes
2	CD2	LFA-3	None	No

Compilation of information from this article, [6], and [Shaw *et al.*, in preparation]. Evidence that the required divalent cation required is Mg^{2+} is based on inhibition by EDTA but not EGTA.



1. Conjugate formation between DPw2-specific clone 8.2 subsets of peripheral blood mononuclear leucocytes. Mono- ear cells from a DPw2-donor were prepared by density ration of fresh peripheral blood, fractionated by adherence astic, and then the non-adherent cells were fractionated by tting with AET-treated SRBC [10]. As internal controls, hoblastoid cell lines (LCL) are included from a DPw2$^+$ and w2-donor.

lies with LFA-1-deficient targets [7], demonstrate t the LFA-1 molecule functions on the effector cell the LFA-3 molecule functions on the target as has n observed with cell-mediated cytotoxicity using ctors and target lines from normal donors [4]. These a strongly suggest that LFA-3 is the ligand on the tar- which is bound by CD2 on the effector since 2/LFA-3 appear to participate in the same pathway bles 1 and 2) and the site of action of CD2 mAb is on effector and that of αLFA-3 is on the target. The othesis that LFA-3 is a ligand for CD2 has been tested l confirmed by studies [Plunkett, Sanders, Selvaraj, w, and Springer, submitted] showing that αLFA-3 ibits: (1) binding of purified CD2 to cells; and (2) cell regation induced by purified CD2. Preliminary dence points to a recently described cell surface mole- , ICAM-1, as a ligand for LFA-1 [8]. Thus, there are different antigen-independent adhesion pathways d by human cytotoxic T-cells; characteristics of those hways are outlined in Table 3.

Subsets of peripheral blood mononuclear leucocytes (PBL) differ in their level of conjugate formation with CTL (Fig. 1). Conjugate formation with antigen-negative adherent PBL is comparable to that with lymphoblastoid cell lines (LCL), while formation with non-adherent subpopulations is consistently lower. Conjugate formation with such adherent cells is inhibited by αLFA-1 more than by CD2 and αLFA-3 mAb (44 per cent versus 0 per cent and 0 per cent inhibition, respectively, in this experiment) while conjugate formation with LCL shows a reciprocal preference (16 per cent versus 33 and 44 per cent inhibition). This and other experiments [Shaw *et al.*, in preparation] indicate that conjugation of CTL with peripheral blood monocytes is principally via the LFA-1 pathway. Thus, conjugates formed between similar CTL and different targets can vary dramatically in their relative utilization of the two pathways [Shaw *et al.*, in preparation]. T-cell rosetting with human erythrocytes illustrates the opposite extreme, since it is mediated via the CD2/LFA-3 pathway [Plunkett *et al.*, submitted for publication].

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