

Identification of a Novel Surface Protein on Activated CD4⁺ T Cells That Induces Contact-dependent B Cell Differentiation (Help)

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Summary

CD4⁺ T lymphocytes provide contact-dependent stimuli to B cells that are critical for the generation of specific antibody responses in a process termed T helper function. The surface structures on activated CD4⁺ T cells that mediate this function are not fully known. We previously reported the isolation of a functionally unique subclone of the Jurkat leukemic T cell line (D1.1) that constitutively expressed contact-dependent helper effector function. To identify T cell surface molecules that mediate contact-dependent T helper function, a monoclonal antibody (mAb), designated 5c8, was generated that inhibits D1.1-mediated B cell activation and immunoprecipitates a novel 30-kD protein structure from surface-iodinated D1.1 cells. Normal CD4⁺ T cells express 5c8 antigen (Ag) transiently after activation by phorbol myristate acetate and phytohemagglutinin with maximal expression 5–6 h after activation and absence of expression by 24 h. In contrast, neither resting nor activated CD8⁺ T cells express 5c8 Ag. In functional studies, mAb 5c8 inhibits the ability of fixed, activated CD4⁺ T cells to induce B cell surface CD23 expression. In addition, mAb 5c8 inhibits the ability of CD4⁺ T cells to direct terminal B cell differentiation driven by pokeweed mitogen. Taken together, these data suggest that 5c8 Ag is a novel, activation-induced surface T cell protein that is involved in mediating a contact-dependent element of the helper effector function of CD4⁺ T lymphocytes.

In a contact-dependent process, termed T helper (Th) function, CD4⁺ T lymphocytes direct the activation and differentiation of B lymphocytes and thereby regulate the humoral immune response by modulating the specificity, secretion and isotype-encoded effector functions of antibody molecules (1–7). The T cell surface molecules that mediate the contact-dependent elements of Th cell function are not fully known.

The process by which T cells help B cells to differentiate has been divided into two distinct phases: the inductive and the effector (8, 9). In the inductive phase, resting T cells contact antigen-primed B cells and this association allows clonotypic TCR-CD4 complexes to interact with Ia/Ag complexes on B cells (5, 10–16). TCR/CD4 recognition of Ia/Ag results in the formation of stable T-B cognate pairs and bidirectional T and B cell activation (17–22). In the effector phase, activated T cells drive B cell differentiation by secreting lymphokines (23, 24) and by contact-dependent stimuli (20, 25–32), both of which are required for T cells to drive small, resting B cells to terminally differentiate into Ig-secreting cells (25, 33, 34).

Although the inductive phase of T cell help is Ag depen-

dent and MHC restricted (5, 10–15, 34, 35), the effector phase of Th function can be Ag independent and MHC nonrestricted (25, 28, 30, 34, 36–43). An additional contrasting feature is that the inductive phase of T cell help often requires CD4 molecules and is inhibited by anti-CD4 mAb (16), whereas helper effector function does not require CD4 molecules (44) and is not inhibited by anti-CD4 mAbs (27, 28, 30, 42). The nonspecific helper effector function is believed to be focused on specific B cell targets by the localized nature of the T-B cell interactions within antigen-specific, cognate pairs (21, 22, 45).

Although terminal B cell differentiation requires both contact-mediated and lymphokine-mediated stimuli from T cells, intermediate stages of B cell differentiation can be induced by activated T cell surfaces in the absence of secreted factors. These intermediate effects on B cells include induction of surface CD23 expression (26, 29, 46), enzymes associated with cell cycle progression (31), and responsiveness to lymphokines (20, 31, 42, 47–49). Although the activation-induced T cell surface molecules that direct B cell activation have not been identified, functional studies have characterized some features of induction and biochemistry. First, T

cells acquire the ability to stimulate B cells 4–8 h after activation (32, 42). Second, the B cell stimulatory activity associated with the surfaces of activated T cells is preserved on paraformaldehyde-fixed cells (20, 26, 31, 42, 49) and on purified membrane fragments (27, 47). Third, the B cell stimulatory activity is sensitive to protease treatment (20, 47, 50). Fourth, the process of acquiring these surface active structures after T cell activation is inhibited by cyclohexamide (42, 47). Although these studies strongly suggest the existence of activation-induced T cell surface proteins that deliver contact-dependent stimuli to B cells, the molecular identities of such structures remain unknown.

We have previously reported the isolation of a CD4⁻ Jurkat subclone (D1.1) that possessed the unique functional potential to activate B cells to express surface CD23 molecules and to support the terminal differentiation of B cells in the presence of lectins (51). Jurkat D1.1 activated B cells from a large number of unrelated donors suggesting that the D1.1 effect was Ag independent and MHC unrestricted. The mechanism of Jurkat D1.1-mediated B cell activation was found to depend on cell-cell contact or close proximity because paraformaldehyde-fixed D1.1 cells, but not secreted factors, possessed the ability to induce B cell CD23. In addition, the effect of D1.1 on B cells was not inhibited by anti-IL-4 antibodies. Further, the effect of D1.1 on B cells was distinct from that of IL-4 because rIL-4 but not D1.1 induced upregulation of B cell surface IgM (sIgM) (51, 52). Taken together, these data suggested that Jurkat D1.1 and activated CD4⁺ T cells shared surface structures that provide contact-dependent elements of T cell help to B cells (51).

In the present work, we generated a murine IgG2a mAb (5c8) that inhibits D1.1-mediated B cell activation and immunoprecipitates a novel 30-kD non disulfide-linked protein from the surface of D1.1. On normal T cells, the 5c8 Ag is transiently expressed on activated CD4⁺ T cells in a manner that requires mRNA and protein synthesis. In functional studies, mAb 5c8 inhibits the ability of T cells to mediate B cell activation and terminal differentiation. Taken together, these data demonstrate that the 5c8 Ag is an important component of the activation-induced T cell surface structures that mediate contact-dependent stimuli for B cell differentiation.

Materials and Methods

Generation and Characterization of 5c8 mAb. Five BALB/c mice were immunized with 2×10^6 D1.1 cells in saline intravenously and then boosted intraperitoneally at five ~2-wk intervals. The sera of these mice were titrated to test for the presence of antibodies that bound preferentially to Jurkat D1.1 (helper clone), versus B2.7 cells (nonhelper clone) by FACS[®] (Becton Dickinson & Co., Mountain View, CA). One mouse, which showed the best differential titer, received a boost of 2×10^6 D1.1 cells intravenously 3 d before fusion. Splenocytes from this mouse were fused with 7×10^7 murine SP2/0 myeloma fusion partner cells as previously described (53). The cell mixture was cultured overnight in DMEM containing 15% FCS before the fusion product was seeded into 360 8-mm wells. Colonies appeared in 220 wells and all were screened by FACS[®] for differential binding to D1.1 and B2.7 cells. A mAb designated 5c8 was found to bind to D1.1 cells and not

B2.7 cells. The 5c8 clone was subcloned multiple times until monoclonality was established. The 5c8 mAb was found to be IgG2a by ELISA (HyClone Laboratories, Logan, UT).

mAbs. The following mAbs were produced by hybridomas available from the American Type Culture Collection (Rockville, MD): OKT4 (anti-CD4), OKT8 (anti-CD8), OKT3 (anti-CD3), W6/32 (anti-MHC class I), and 187.1 (anti-human Ig [Fab]). These mAbs were either used at saturating concentrations of hybridoma supernatants, or purified from ascites fluid on protein A columns (Bio-Rad Laboratories, Richmond, CA). Anti-CD23-PE mAbs and unlabeled anti-CD69 were purchased from Becton Dickinson & Co. FITC-labeled anti-IgM was purchased from Tago Inc. (Burlingame, CA). KOLT-4 (anti-CD28) was purchased from Accurate Chemical & Scientific Corp. (Westbury, NY).

Cytofluorographic Analysis. Approximately 10^5 cells were incubated with saturating concentrations of the indicated mAbs for 45 min at 4°C in the presence of 80 µg/ml heat-aggregated human IgG (International Enzyme, Fallbrook, CA). Cells were washed to remove unbound mAb before incubation with F(ab)₂ goat anti-mouse Ig secondary antibody coupled to fluorescein (Cappel Laboratories, Cochranville, PA). For two-color analysis, cells were reacted with the indicated directly coupled FITC- or PE-conjugated mAb for 45 min at 4°C in the presence of aggregated human IgG. Before analysis, cells were washed and resuspended in PBS. Fluorescence intensity was measured on a FACScan[®] cytofluorograph (Becton Dickinson & Co.). In experiments involving coculture of B cells with Jurkat clones, the Jurkat cells were excluded from the analysis of B cell fluorescence by gating on the distinct population of cells with low forward and side light scatter. In experiments with PMA- and PHA-activated cells, dead cells were excluded from analysis by treatment with propidium iodide and electronic FACS[®] gating.

Cell Lines. The following cell lines were from the American Type Culture Collection: Jurkat, CEM, PEER, MOLT-IV, K562, Ramos, Raji, and U937. BA is an EBV-transformed B cell line that has been previously reported (54). H9 is available from the HIV Repository (Rockville, MD).

Isolation of Cell Populations. PBL were obtained from the freshly drawn blood of healthy volunteers by centrifugation on Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO). T cells were positively selected with neuraminidase-treated sheep erythrocytes. CD4⁺CD8⁻ and CD4⁻CD8⁺ T cell subsets were isolated by anti-CD8 or anti-CD4 mAb treatment, respectively, followed by complement-mediated lysis as previously described (16). B cells were derived from the population of cells that did not pellet through Ficoll-Hypaque after two rounds of rosetting with neuraminidase-treated sheep erythrocytes.

B cells were further purified by either density centrifugation or by positive selection on an anti-Ig column, as indicated in the text or figure legends. In the first method, E⁻ cells were cultured overnight in polystyrene flasks (37°C, 5% CO₂) to adherence deplete macrophages. These non-T cell, nonmacrophage cells were fractionated into high- and low-density fractions in a discontinuous 30%/50%/100% percoll gradient by centrifugation at 2,300 rpm for 12 min. High-density cells were obtained from the 50/100% interface and low-density cells from the 30/50% interface (55). The high-density (resting) cells were typically 60–80% CD20⁺, 55–80% IgM⁺, and <5% CD3⁺ and <5% CD23⁺ (background). In other experiments (where indicated) B cells were purified by sephadex G-200 anti-F(ab)₂ Ig affinity chromatography into sIg⁺ cells, as has been described (16, 56). The sIg⁺ populations were typically <5% CD3⁺, <10% CD2⁺, and >90% CD20⁺ when analyzed by FACS[®].

SDS-PAGE. Jurkat clones were iodinated by the lactoperoxidase method, solubilized in 1% NP-40, 15 mM Tris-buffered saline containing iodoacetamide, and 1 mM PMSF. The cell lysates were reacted with protein A-4B Sepharose beads (Pharmacia, Uppsala, Sweden) that were coated with mAb 187.1 (anti-human F[ab]Ig) and ~100 ng of the indicated mAb. After washing the beads to remove nonspecifically bound proteins, the precipitated proteins were denatured by heating in SDS in the presence or absence of 2-ME. The denatured proteins and prestained molecular weight markers (Bio-Rad Laboratories) were subjected to electrophoresis through 12.5% polyacrylamide in 12-cm gels (Bio-Rad Laboratories), and fixed, dried gels were used to expose x-ray film (Kodak, Rochester, NY).

Mitomycin C and Paraformaldehyde Treatments. Jurkat cells (10^7 /ml) were treated with 50 μ g/ml mitomycin C (Sigma Chemical Co.) for 60 min at 37°C. The mitomycin-treated Jurkat cells were washed twice, resuspended in mitomycin free media, and then cultured for 45–60 min at 37°C. The cells were washed two additional times and then added to the B cell cultures. In fixation experiments, T cells were treated with freshly made 0.5% paraformaldehyde for 5 min, quenched with 0.2 M L-lysine, and washed five times before addition to cultures of B cells.

T Cell Activation. In experiments studying expression of 5c8 Ag, resting T cells were cultured in the presence or absence of 10 ng/ml PMA (Sigma Chemical Co.) and 10 μ g/ml PHA (Sigma Chemical Co.). In experiments studying the metabolic requirements for 5c8 Ag expression, T cells were activated in the presence of 100 μ M cyclohexamide (Sigma Chemical Co.) or 10 μ g/ml actinomycin D (Sigma Chemical Co.).

In experiments studying the induction of CD23 expression on high-density B cells by activated T cells, the mAbs OKT3 or OKT4 were immobilized on the surfaces of 24-well culture plates by incubation of 10 μ g/ml of mAb in normal saline for 1 h. Control wells were incubated in saline containing no mAb. After washing unbound mAb or saline, T cells were cultured in the control or mAb-coated plates at 2×10^6 cells/well in the presence or absence of 10 ng/ml phorbol dibutyrate (PDB)¹ (Sigma Chemical Co.) for 6 h. The cells were removed by vigorous pipetting, washed, and fixed with 0.5% paraformaldehyde as described above before culture at the indicated ratio with 2×10^5 high-density, percoll-isolated, resting B cells for 18 h. B cell CD23 expression was determined by two-color FACS[®] as described above.

Assays of B Cell Activation and Differentiation. In experiments measuring the induction of B cell surface CD23 expression, 2×10^5 high-density B cells were added to the indicated number of Jurkat cells or T cells in 200 μ l of IMDM-10% FCS in round-bottomed microtiter wells (Nunc, Roskilde, Denmark) and assayed for CD23 expression after 18–24 h.

The measurement of plaque-forming colonies (PFC) was a modification of the reverse hemolytic plaque assay (16). Briefly, 2.5×10^5 B cells were cultured with varying numbers of mitomycin C-treated Jurkat cells or untreated freshly isolated, autologous T cells for 6 d in the presence or absence of a 1:400 dilution of PWM (Gibco Laboratories, Grand Island, NY). The cells were washed twice and resuspended in HBSS. From an appropriate dilution, 50 μ l of cultured cell suspension was mixed with: 10 μ l of an 11% solution of SRBC that had been coated with rabbit anti-human Ig by chromic chloride, 10 μ l of diluted rabbit anti-human Ig, and 10 μ l of guinea pig complement. These mixtures were introduced

into duplicate glass chambers and cultured for 1 h at 37°C. Plaques were counted using a dissecting microscope and expressed as PFC/ 10^6 B cells.

Results

To characterize cell surface proteins on activated CD4⁺ T cells that mediate helper effector function, mice were immunized with the D1.1 clone of Jurkat that possessed contact-dependent helper effector function (51). mAbs were generated and hybridoma supernatants were screened for differential binding to the D1.1 clone and a nonhelper Jurkat clone, B2.7. A murine IgG2a mAb, termed 5c8, was identified that bound specifically to the surface of D1.1 cells and not to the surface of the nonhelper, B2.7 cells (Table 1). Because the mAb 5c8 bound specifically to the helper Jurkat clone, D1.1, the mAb 5c8 was studied in further detail. The mAb 5c8 did not bind to a variety of other cell lines, including the T cell leukemia lines CEM, H9, Molt-4, and Peer; the B cell-derived cell lines BA, Raji, or Ramos; the myelomonocytic cell line U937; or the erythroleukemia cell line K562 (Table 1).

To assess whether mAb 5c8 reacted with a molecule that was functionally relevant to the helper capacity of the Jurkat clone D1.1, the effect of mAb 5c8 was studied in assays of D1.1-induced CD23 expression on B cells. The mAb 5c8 potently inhibited Jurkat D1.1-induced B cell activation (Fig. 1). In contrast, the isotype control mAb, W6/32 (Fig. 1),

Table 1. The Expression of 5c8 Ag on Cell Populations and Cell Lines

Cell lines	Resting	Activated
Jurkat D1.1	+	+
Jurkat B2.7	-	-
CEM	-	-
H9	-	ND
Molt-4	-	-
PEER	-	-
BA	-	ND
Raji	-	ND
Ramos	-	ND
U937	-	-
K562	-	ND
Cell populations		
T cells	-	+
B cells	-	-
Monocytes	-	-

These data derive from FACS[®] analyses of mAb 5c8 binding to the indicated cell lines or cell populations. The presence of mAb 5c8 binding was determined relative to FACS[®] staining of appropriate positive and negative control mAbs for each cell line or population.

¹ Abbreviations used in this paper: PDB, phorbol dibutyrate; PFC, plaque-forming colonies.

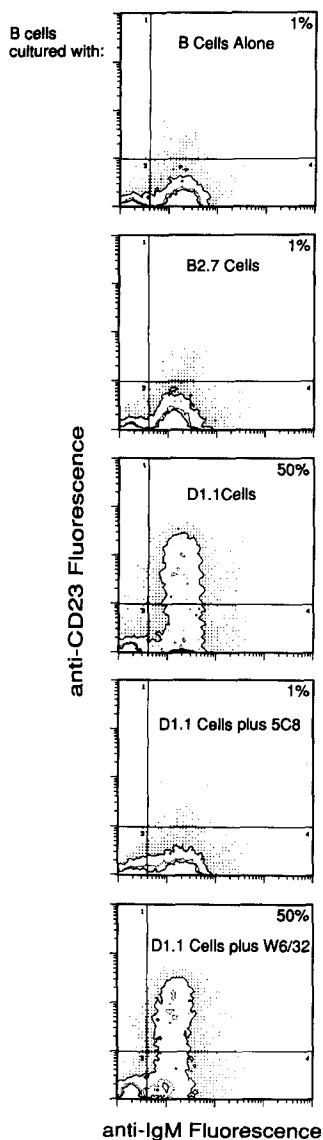


Figure 1. mAb 5c8 inhibits Jurkat D1.1-induced CD23 expression by B lymphocytes. Shown are two-color FACS[®] analyses of adherence-depleted, high-density B cells after 24 h of culture alone or with the B2.7 or D1.1 Jurkat clones using anti-IgM-FITC (on the x-axis) and anti-CD23-PE (on the y-axis). The number in the upper right hand corner of the FACS[®] tracings represents the percentage of IgM⁺ cells that expressed CD23. The mAbs were present at 1 μ g/ml. The murine IgG2a mAb W6/32 recognizes a monomorphic determinant on class I MHC molecules.

did not inhibit D1.1-mediated B cell activation. The data presented here suggested that the 5c8 Ag played a critical role in the helper effector function of D1.1 cells.

To biochemically characterize the Ag recognized by mAb 5c8, immunoprecipitations were performed with mAb 5c8 or control mAbs that recognized class I MHC (W6/32) or CD28 (Kolt-4) on cell lysates of surface-iodinated Jurkat D1.1 cells and control, nonhelper Jurkat B2.7 cells that lacked surface mAb 5c8 binding. The mAb 5c8 immunoprecipitated a protein that migrated on SDS/PAGE at 30 kD from lysates of the helper clone D1.1 but not from the control B2.7 lysates (Fig. 2). The protein species immunoprecipitated by mAb 5c8 was not affected by reduction with 2-ME, suggesting that the 30-kD band was neither a disulfide-linked homodimer nor disulfide linked to another protein that was not accessible to iodination (Fig. 2). In contrast, the control, anti-

CD28 mAb, KOLT-4 immunoprecipitated an 88-kD band in the absence of 2-ME and a 44-kD band in the presence of 2-ME that is consistent with published reports (57) and with the interpretation that this structure is a disulfide-linked homodimer (Fig. 2). The control mAb W6/32 precipitated a non-disulfide-linked heterodimer of 43- and 12-kD proteins (Fig. 2). These data suggested that the mAb 5c8 recognized a 30-kD non-disulfide-linked protein species from the surface of D1.1 cells.

The next series of experiments characterized the expression of 5c8 Ag by normal lymphoid cells. The binding of mAb 5c8 or a variety of control mAbs was studied by FACS[®] on freshly isolated, T and B lymphocytes, monocytes, and PMA- and PHA-stimulated T cells. Although resting T or B lymphocytes or monocytes did not express 5c8 Ag (Table 1, Fig. 3), a subset of activated T cells was found to express 5c8 Ag, 5 h after activation with PMA and PHA (Fig. 3).

To characterize the kinetics and cellular distribution of 5c8 Ag expression, the binding of mAb 5c8 to T cells was studied by FACS[®] at various intervals after T cell activation. The CD69 molecule, which is a 32/28-kD disulfide-linked heterodimer, was selected as a control because it is known to be induced rapidly on virtually all T cells after T cell activation (58, 59). Whereas 5c8 Ag was absent from resting T cells and was expressed on a subset of T cells after activation, in contrast, a variable, but low level of CD69 expression was present on resting T cells, and high-level CD69 expression was induced by activation on the entire T cell population (Fig. 3). The kinetics of expression further distinguished 5c8 Ag from CD69 because mAb 5c8 binding was significant 3 h after activation, peaked at 6 h, and returned to baseline (no binding) after 24 h, whereas high-level CD69 was induced 1 h after activation (58, 59, and data not shown) and persisted for >24 h (Fig. 4). The data presented here distinguish the 5c8 Ag from CD69 both by the cellular distribution of their expression and by the kinetics of their upregulation after activation.

To determine if mRNA or protein synthesis were required for 5c8 Ag expression, T cells were stimulated with PMA and PHA in the presence or absence of actinomycin D or cyclohexamide and the expression of 5c8 Ag and CD69 was compared. The expression of 5c8 Ag was inhibited by either actinomycin D or cyclohexamide treatment (Fig. 3). In contrast, CD69 was upregulated by activation (although to a lesser extent) despite the presence of 10 μ g/ml actinomycin D or 100 μ M cyclohexamide (Fig. 3), as has been reported previously (58–60). These data suggested that the expression of the 5c8 Ag after T cell activation depends on transcription of mRNA and de novo protein synthesis.

To characterize the subset of T cells that expressed 5c8 Ag after activation, CD4⁺CD8⁻ or CD4⁻CD8⁺ T cell populations were isolated by anti-CD8 or anti-CD4 mAb treatment, respectively, followed by complement depletion. The CD4⁺CD8⁻ or CD4⁻CD8⁺ populations were activated with PHA and PMA and studied for 5c8 Ag or CD69 expression by FACS[®]. After activation, 5c8 Ag expression was induced exclusively on CD4⁺ T cells and not on CD8⁺ T

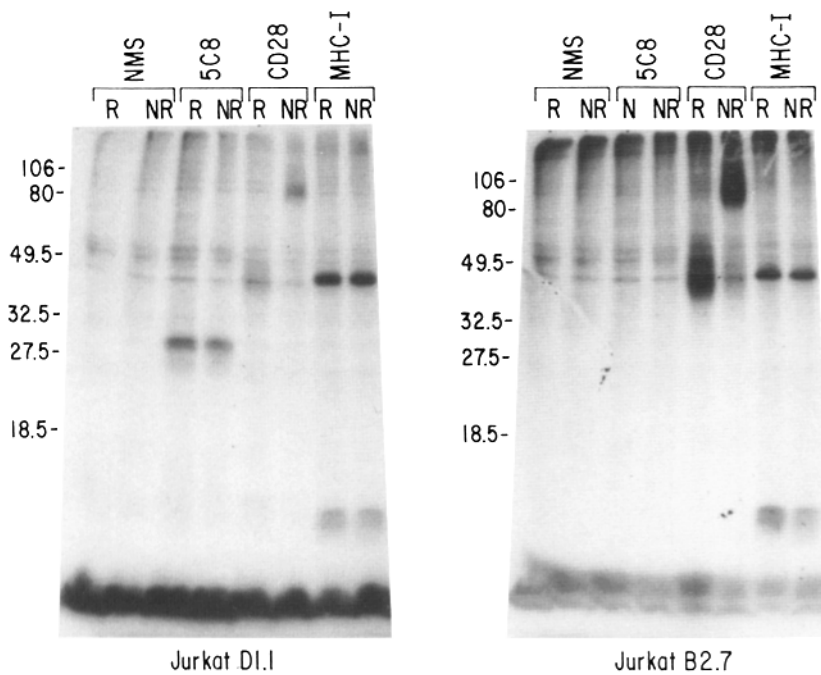


Figure 2. SDS/PAGE analysis of surface proteins immunoprecipitated by mAb 5c8 and control mAbs. Shown are autoradiograms of immunoprecipitates with mAb 5c8 or control mAbs from cell lysates of surface-iodinated Jurkat D1.1 or Jurkat B2.7 cells that were separated on 12.5% polyacrylamide in the presence (reduced, R) or absence (nonreduced, NR) of 2-ME. mAbs shown are anti-CD28 (KOLT-4) and anti-MHC class I (W6/32). Molecular mass markers represent the migration of prelabeled standards. NMS, normal mouse serum.

cells, despite the fact that CD4⁺ and CD8⁺ cells expressed similar levels of CD69 after activation (Fig. 4). Taken together, these data demonstrated that 5c8 Ag expression is restricted to activated CD4⁺ T cells.

To evaluate the role of 5c8 Ag in Th function mediated by normal T cells, the effect of mAb 5c8 was studied on the ability of activated T cells to induce small resting B cells to express surface CD23 molecules. Freshly isolated CD4⁺ T cells were cultured on surfaces that were either uncoated, or coated with anti-CD3 (OKT3) or control, anti-CD4 (OKT4), mAbs in the presence of PDB, and then fixed with paraformaldehyde. These fixed T cells were studied for B cell-activating capacity in the presence of soluble mAbs 5c8 or OKT4. The mAb OKT4 was selected as a control for these experiments because OKT4 is an isotype-matched control mAb that reacts with CD4⁺ T cells but does not affect T-B interactions (16). CD4⁺ T cells activated by either PDB or immobilized OKT3 alone, before fixation, induced CD23 expression on 6% and 13% of B cells, respectively (Table 2). T cells stimulated with both anti-CD3 plus PDB before fixation activated 32% of B cells, which was similar to the potency of paraformaldehyde-fixed D1.1 cells (36%) (Table 2). The mAb 5c8 completely inhibited the ability of D1.1 cells to induce B cell activation and inhibited the activating ability of CD4⁺ T cells by 63% (Table 2). These data are representative of four similar experiments and suggest that 5c8 Ag on activated CD4⁺ T cells plays an important role in B cell activation.

We next studied the effect of mAb 5c8 on terminal B cell differentiation driven by normal human T cells. In these experiments, CD4⁺ T cells were cultured with autologous, column-isolated B cells in the presence of PWM, and the

number of Ig-secreting B cells (PFC) was measured by reverse hemolytic plaque assay. The mAb 5c8, but not OKT4, inhibited the CD4⁺ cell-driven PFC response (Table 3). Taken together, these data demonstrate that the 5c8 Ag mediates a contact-dependent aspect of the helper effector function of activated CD4⁺ T cells.

Discussion

In the present work, a functionally unique subclone of the Jurkat leukemic line (D1.1) with constitutive, contact-dependent helper function was used to generate a murine mAb, designated 5c8, that inhibited D1.1-induced B cell activation. The mAb 5c8 recognized a unique protein species on D1.1 cells that was not disulfide linked and migrated at 30 kDa on SDS/PAGE. On normal lymphoid cells, the expression of 5c8 Ag was restricted to a subset of T lymphocytes after activation. The activation-induced expression of 5c8 Ag on T cells required transcription of mRNA and de novo protein synthesis. The 5c8 Ag was found to be transiently expressed on activated T cells with peak expression at 6 h and loss of expression by 24 h. The expression of 5c8 Ag was restricted exclusively to activated CD4⁺ T cells. In functional studies on normal T cells, the mAb 5c8 inhibited the ability of fixed, activated T cells to induce B cell CD23 expression. In addition, mAb 5c8 inhibited the ability of normal CD4⁺ T cells to direct B cell differentiation. Taken together, these data demonstrate that the 5c8 Ag is a novel activation-induced surface protein expressed exclusively on activated CD4⁺ T cells that is involved in mediating a contact-dependent element of Th function.

The tissue distribution, kinetics of expression, and bio-

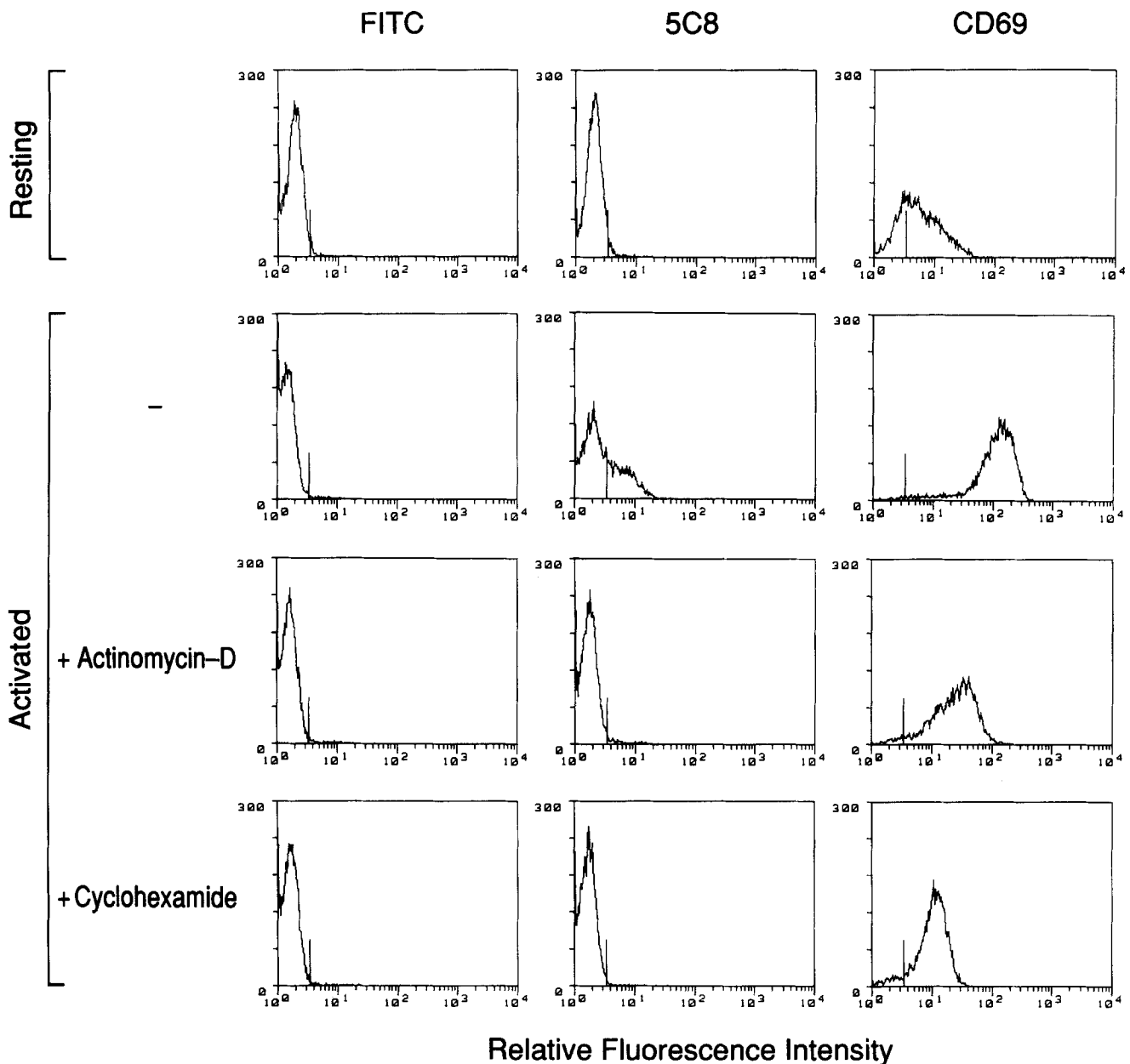


Figure 3. Effects of T cell activation and metabolic inhibitors on the expression of 5c8 Ag. Shown are FACS[®] histograms of resting and activated T cells using mAb 5c8 or anti-CD69 as indicated. T cell activation was induced by PMA (10 ng/ml) and PHA (10 μ g/ml) for 5 h. Where indicated, activation was performed in the presence of actinomycin D (10 μ g/ml) or cyclohexamide (100 μ M).

chemistry of the 5c8 Ag distinguished the 5c8 Ag from other known surface proteins induced by T cell activation. First, all other known human T cell activation markers (e.g., CD69, CD25, Ia) are expressed by both CD4⁺ and CD8⁺ T cells, whereas the 5c8 Ag is expressed exclusively by CD4⁺ T cells. In the rat, an activation molecule designated OX-40 is restricted to CD4⁺ T cells, but appears to be distinct from 5c8 Ag by virtue of its molecular mass and kinetics of expression (see below) (61). Second, the kinetics of 5c8 Ag expression after T cell activation were distinct from that of other T cell activation molecules. Whereas 5c8 Ag was maximally

expressed 6 h after activation and absent 24 h after activation, CD25 (62), Ia (63, 64), and the 32-kD form of CD27 (65) are induced 18 h or more after activation. In addition, CD69 and OX-40 expression persists for >24 h. Third, the 5c8 Ag is a 30-kD, non-disulfide-linked species that is distinct from CD69 (28/32-kD disulfide-linked heterodimer [59]) and OX-40 (50 kD). Taken together, these data suggest that the 5c8 Ag was distinct from other known T cell activation molecules.

The 5c8 Ag was also distinguished from other T cell surface molecules that are known to play roles in T-B interac-

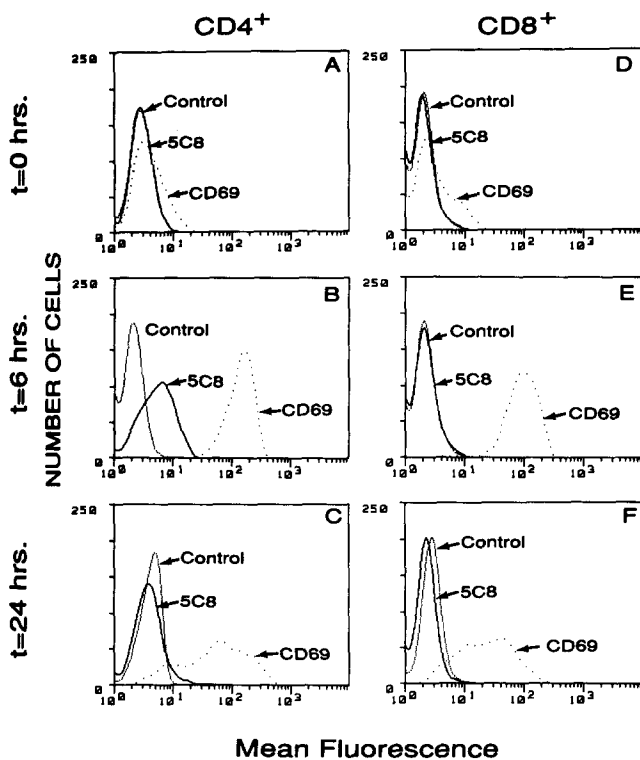


Figure 4. Kinetics of expression of 5c8 Ag on isolated CD4⁺ or CD8⁺ T cell subsets. Shown are fluorescence histograms of CD4⁺ cells or CD8⁺ cells at the indicated time points after freshly purified T cell subsets were activated with PHA (10 μg/ml) and PMA (10 ng/ml). Solid line, 5c8 binding; dashed line, IgG2a control; dotted line, anti-CD69.

tions by several aspects of their tissue distribution and biochemistry. First, 5c8 Ag was induced by T cell activation but was not expressed on resting cells. In contrast, CD4, CD2, CD5, CD28, LFA-1, ICAM-1, CD45RO, and 6C2, which interact with B cell surface ligands (66–74) are expressed on resting T cells (73–77). Second, the specific expression of 5c8 Ag on activated T lymphocytes and not on B cells, monocytes, or the panel of cell lines (Table 1) distinguished 5c8 Ag from ICAM-1, CD4, CD5, LFA-1, CD2, and 6C2 molecules, which are also expressed on either monocytes, B cells, or certain of the cell lines (data not shown). Third, the expression of 5c8 Ag was restricted to CD4⁺ T cells, whereas CD2, CD5, CD28, LFA-1, ICAM-1, CD45RO, and 6C2 are expressed on CD8⁺ as well as CD4⁺ cells (73–77). Fourth, the 30-kD protein precipitated by mAb 5c8 is unlike any of these other proteins (73–77). Finally, 5c8 Ag was distinct from these other molecules because mAb 5c8 was identified by its ability to inhibit the helper effector function mediated by Jurkat D1.1.

Because mAb 5c8 inhibits the contact-dependent helper effects of Jurkat D1.1 and fixed, activated CD4⁺ T lymphocytes, it is likely that 5c8 Ag mediates a B cell-activating function by interacting with a ligand (or “counter-receptor”) on the surfaces of B cells. The interaction of 5c8 Ag with a B cell counter-receptor may mediate helper function either by providing additional adhesive forces to T-B pairs, transducing a stimulatory signal to B cell cytoplasm, or by a combination of these mechanisms. Regardless of the precise mechanism, the transient expression of 5c8 Ag may provide a

Table 2. The Effect of mAb 5c8 Treatment on B Cell Surface CD23 Induction Mediated by Paraformaldehyde-fixed, Activated CD4⁺ T Cells

Resting B cells cultured with:	Soluble mAbs		
	Media	5c8	OKT4
No T cells	2		
B2.7	4		
D1.1 (1:1)	78	2	78
D1.1 fixed	36	2	32
	Phorbol Immobilized mAb		
CD4 ⁺ T (fixed)	Control	None	2
CD4 ⁺ T (fixed)	Control	OKT3	13
CD4 ⁺ T (fixed)	Control	OKT4	3
CD4 ⁺ T (fixed)	+ PDB	None	6
CD4 ⁺ T (fixed)	+ PDB	OKT3	32
CD4 ⁺ T (fixed)	+ PDB	OKT4	4

Shown are the percentages of IgM⁺ B cells that expressed CD23 by two-color FACS[®] analysis after B cells were cultured alone or in the presence of a 4:1 ratio (T/B) of paraformaldehyde-fixed Jurkat D1.1 cells or CD4⁺ T cells that had been stimulated with 10 ng/ml PDB alone or in the presence of either immobilized anti-CD3 (OKT3) or anti-MHC-I (W6/32) mAbs, as indicated. The IgG2a mAbs, 5c8 and OKT4, were present in solution at 5 μg/ml. Also shown is the effect of a 1:1 ratio of Jurkat D1.1 and B2.7 without paraformaldehyde fixation.

Table 3. *The Effect of mAb 5c8 Treatment on the Induction of Antibody-forming Cells*

T cells	B cells	PWM	mAb	PFC		
				Exp. 1	Exp. 2	Exp. 3
	B			120	240	600
	B	PWM		240	600	4,800
CD4 ⁺ T				240	120	180
CD4 ⁺ T	B			2,580	780	ND
CD4 ⁺ T		PWM		3,840	240	60
CD4 ⁺ T	B	PWM	Media	149,760	85,200	25,800
CD4 ⁺ T	B	PWM	5c8	58,000	4,680	9,000
CD4 ⁺ T	B	PWM	OKT4	143,520	103,200	30,960

Shown are the results of three separate experiments on unrelated donors in which CD4⁺ T cells were cultured in a 0.6:1 ratio with autologous, anti-Ig column-isolated B cells in the presence or absence of PWM. The number of PFC per 10⁶ B cells was measured by reverse hemolytic plaque assay. The mAbs 5c8 and OKT4 were present at 500 ng/ml, except in Exp. 1, in which OKT4 was present at 1 µg/ml.

molecular solution to limiting nonspecific B cell activation. We envision that the transient expression of 5c8 Ag in the localized milieu of antigen-specific cognate T-B pairs may channel the antigen/MHC-unrestricted activating function of 5c8 Ag to appropriate B cell targets. The kinetics of expression and downregulation of 5c8 Ag are shared by the endothelial cell, activation-induced, cell surface mediator of leukocyte and lymphocyte binding, ELAM-1 (78). This similarity might indicate that the strategy of using transient expression to affect localized intercellular interactions may be shared by 5c8 Ag, ELAM-1, and potentially other, yet uncharacterized, surface molecules that transmit potent signals to other cells by direct contact.

The CD4 molecule identifies the population of T cells that contains precursors of T cells with helper function (4). However, the CD4⁺ subset is functionally heterogeneous and contains cytotoxic and suppressor cells in addition to helper cells (79, 80). The fact that 5c8 Ag is involved in helper function suggests that 5c8 Ag may correlate more closely with

the helper phenotype than CD4 expression. The heterogeneous distribution of 5c8 expression on activated CD4⁺ cells suggests that functional subsets of CD4⁺ T cells might be distinguished by their level of 5c8 expression. For example, it will be of interest to determine the functional potential of 5c8⁻ and 5c8⁺ CD4⁺ T cells with respect to helper or cytotoxic activity.

T cell helper effector function is a complex process resulting in B cell responsiveness (19, 47-49), regulation of isotype switching (81), and somatic hypermutation (82). The fact that T cells interact with B cells by a number of cell-cell interactions as well as by secreting various lymphokines suggests that individual signals or certain combinations of signals may regulate specific aspects of B cell differentiation. The fact that mAb 5c8 inhibits a contact-dependent aspect of T cell helper function provides a means of further dissecting the processes by which CD4⁺ T cells regulate the humoral immune response.

We thank Drs. Ned Braunstein and Paul Rothman for critical reading of this manuscript, and Ruth Croson and Eva Glickman for excellent technical assistance.

This work was supported by National Institutes of Health grants PO1-AI-26886 and RO-1-AI-14969. M. J. Yellin was supported by an NIH Immunology Training Grant (AI-07132).

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Received for publication 21 October 1991 and in revised form 10 January 1992.

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