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A monoclonal antibody specific for mouse dendritic cells

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ABSTRACT **Dendritic cells (DC) are a small subpopulation of lymphoid cells with distinctive cytologic features, surface properties, and functions. This report describes the DC-specific antibody (Ab) secreted by clone 33D1. Rat spleen cells immune to mouse DC were fused to the P3U myeloma. Hybrid culture supernatants were screened simultaneously against DC, a macrophage (MΦ) cell line, and mitogen-stimulated lymphoblasts. 33D1 Ab specifically killed 80–90% of DC from spleen and lymph node, but no other leukocytes, including Ia^+ and Ia^- MΦ (Ia, *I*-region-associated antigen). Quantitative binding studies with ^3H-labeled 33D1 Ab showed that DC had an average of 14,000 binding sites per cell. Binding to DC was inhibited with Fab fragment of 33D1 Ab but not with a panel of other monoclonal antibodies, including anti-Ia Ab. Adherence and flotation procedures that enriched for DC enriched for ^3H-labeled 33D1 Ab binding in parallel. 33D1 antigen was not detectable on: MΦ from spleen, peritoneal cavity, and blood; three MΦ cell lines; lymphocytes; granulocytes; platelets; and erythroid cells. DC continued to express the 33D1 antigen after 4 days in culture, whereas MΦ and lymphocytes did not acquire it. Quantitative and autoradiographic studies confirmed that spleen and lymph node suspensions contain less than 1% DC. We conclude that 33D1 Ab detects a stable and specific DC antigen and can be used to monitor DC content in complex lymphoid mixtures.**

Dendritic cells (DC) are morphologically distinct cells discovered in adherent populations from mouse lymphoid organs (1). DC can be highly enriched on the basis of low buoyant density, adherence to glass or plastic, and lack of Fc fragment receptors (2). DC express abundant *I*-region-associated antigen (Ia Ag) and lack the characteristic surface markers of B cells, T cells, macrophages (MΦ), and granulocytes (refs. 3 and 4, reviewed in ref. 5). DC are also functionally distinct. For example, DC are 100-fold more effective than other leukocytes in stimulating allogeneic and syngeneic mixed leukocyte reactions (6, 7) and function as potent accessory cells during the generation of cytotoxic lymphocytes (8) and oxidative mitogenesis (9, 10).

A DC-specific antibody (Ab) would be a valuable probe for further *in vitro* and *in vivo* studies. Attempts to generate such a reagent by injecting mouse DC into rabbits failed because the principal reactivity was directed to Ia and H-2 Ag (unpublished data). In this communication, we have used hybridoma technology to generate the DC-specific Ab, clone 33D1. Specificity was monitored by cytotoxicity assays as well as quantitative binding and autoradiographic studies with radiolabeled Ab. Expression of the 33D1 Ag in mouse lymphoid suspensions precisely paralleled the distribution of DC as previously assessed by cytologic (1–3) and functional (6) assays.

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MATERIALS AND METHODS

Animals. (DBA/2 × BALB/c) F_1/Tru, (A × C57BL/6) F_1/Tru, C57BL/6 Tru, (BALB/c × C57BL/6) F_1/Tru, and C3H/J, C3H.OH/J, and Swiss mice were obtained from the Trudeau Institute (Saranac Lake, NY), The Jackson Laboratory, Taconic Farms (Germantown, NY), and The Rockefeller University (New York). Sprague female rats were from Charles River Breeding Laboratories.

Antibodies. Rabbit antiserum to rat Ig (Cappel Laboratories, Cochranville, PA) was affinity purified on Sepharose-rat-Ig and digested to $F(ab')_2$ fragments as described (4). Rabbit antiserum to mouse Ig was from Cappel Laboratories. A number of monoclonal antibodies of defined specificity (4) were employed: B21-2 and 2E8 are anti-I-A reagents; B25-1 is anti-H-2D; 2.4G2 identifies the Fcγ receptor; B5-3 is anti-Thy-1; 1.21 J reacts with the "mac-1" Ag on MΦ and granulocytes; and 2.D2C and 2.6 react with Ag found on DC as well as other cell types.

Cells. Suspensions of spleen, lymph node, and thymus were prepared by teasing and disruption on a stainless steel sieve. Bone marrow was flushed from femurs, peritoneal cells were harvested from the abdominal cavity, and blood mononuclear leukocytes were separated on dense Ficoll/Hypaque. DC were purified from spleen and lymph node by a four-step procedure that involved: flotation on dense albumin columns, adherence to glass or plastic, overnight culture (during which DC lose the capacity to adhere), and removal of MΦ by rosette formation with Ab-coated erythrocytes (EA) or readherence as described (2, 7). MΦ were purified from spleen, node, peripheral blood, and peritoneal cavity by adherence (4). The J774, P388D1, and RA264.7 MΦ cell lines were the gift of J. Unkeless and were maintained in α modified minimal essential medium containing 10% fetal calf serum.

Preparation of Hybridoma Ab. A 300- to 400-g rat was immunized with 3×10^6 DC in complete Freund's adjuvant subcutaneously in the footpads and back. The rat was boosted intravenously with 3×10^6 DC on days 30, 60, and 90. Immune spleen cells were fused with the mouse myeloma line P3U-1 at a ratio of 4:1 three days after the last DC boost, according to the method of Kohler and Milstein (11). Supernatants were assayed by using an indirect binding assay on low-density spleen adherent cells [LODAC, a mixture of DC and MΦ (2)], the J774 MΦ cell line, and phytohemagglutinin-stimulated lymphoblasts (12). T lymphoblasts, which are rich in Ia, were required to detect clones secreting anti-Ia antibodies. Test cells (10^4) were attached to Terasaki dishes (Falcon Plastics, Cockeysville, MD) coated with poly(L-lysine) [poly(L-Lys), 50 μg/ml; Sigma] and

Abbreviations: DC, dendritic cell(s); MΦ, macrophage(s); Ag, antigen; Ia, *I*-region-associated Ag; Ab, antibody; EA, erythrocytes coated with Ab; C, complement; LODAC, low-density adherent cells.

then exposed sequentially to hybridoma supernatants and ^{125}I-labeled F(ab')$_2$ anti-rat Ig at 10 μg/ml and 10–20 μCi/μg (1 Ci = 3.7 × 10^{10} becquerels). Supernatants binding preferentially to DC were identified by autoradiography with Kodak XR-1 film exposed at −70°C for 12–24 hr with image intensifier screens (Lightning Plus, Du Pont). Selected hybridomas were then cloned on agar and ascites was produced by injecting 5–10 × 10^6 cells into mice primed with pristane (Aldrich) and irradiated [600 rads (6 grays)].

Purification, Digestion, and Radiolabeling of 33D1 Ab. The DC-specific and cytotoxic reagent 33D1 was purified from ascites by two precipitations with 45% saturated ammonium sulfate followed by chromatography on DEAE-cellulose (DE-52, Whatman) developed with 0.0175 M sodium phosphate, pH 6.3. Fab fragments were obtained by digestion with 1% papain (Sigma) in 0.1 M sodium acetate (pH 5.5)/10 mM 2-mercaptoethanol/1 mM EDTA for 12 hr at 37°C. The digestion was monitored by sodium dodecyl sulfate/polyacrylamide gel electrophoresis under reducing conditions (Fig. 1). 33D1 was labeled with boro[^{3}H]hydride (New England Nuclear) by reductive methylation to a level of 2–5 μCi/μg exactly as described by Tack *et al.* (13). The 33D1 Ab was inactivated if radioiodinated by chloramine-T, IODO-GEN, lactoperoxidase and glucose oxidase, or Bolton–Hunter procedures under conditions in which other monoclonal antibodies were successfully labeled (4). An anti-Ia Ab, 2E8, was also tritiated, although a much lower specific activity (0.1 μCi/μg) was sufficient.

Cytotoxicity Assays. All cytotoxicity assays were performed with excess Ab (saturating concentration for 33D1-mediated killing was 1–2 μg/ml). Cells at 5–10 × 10^6 per ml were mixed with equal volumes of Ab and rabbit complement (C) (absorbed with agarose, 80 mg/ml) for 60 min at 37°C. Cytotoxicity in suspension was assessed by trypan blue exclusion. Killing of DC or MΦ on adherent monolayers was monitored as described in the text.

Quantitative Binding Studies with Radiolabeled Ab. Labeled Ab was added on ice for 60 min to cells in suspension, adherent to tissue culture surfaces, or attached to 16-mm wells coated with poly(L-Lys). Unless otherwise stated, the binding mixtures contained saturating concentrations of labeled Ab, 5% newborn calf serum, 0.02% sodium azide, and saturating levels of nonlabeled 2.4G2 anti-Fc receptor Ab to block Fc-mediated binding (14). After 60 min, the cells were rapidly washed (2–5 min) and then lysed in 0.1 M NaOH, and radioactivity was measured by using Hydrofluor (National Diagnostics, Somerville, NJ) as scintillant. Bound ^{3}H-labeled 33D1 (^{3}H-33D1) dissociated from DC slowly (10–15% per 15 min). The amount of binding to cells in suspension was identical to that bound by cells attached to poly(L-Lys). Background binding (100–350 cpm per assay) was determined by omitting cells or blocking with a 20- to 30-fold excess of unlabeled Ab.

Binding assays were also used to quantitate the MΦ and Ia content of adherent cell preparations. ^{125}I-Labeled 1.21J (anti-mac-1) and ^{125}I-labeled B21-2 (anti-I-$A^{b,d}$) were used as described (4).

Autoradiography. ^{3}H-33D1 was applied to cells adherent, or attached [poly(L-Lys)], to coverslips as in quantitative binding assays. Coverslips were washed, blow dried, fixed in methanol, attached to slides, dipped in Ilford L4 emulsion (Polysciences, Warrington, PA) diluted 1:3 in water, and exposed 10–20 days prior to development in D19 (Eastman).

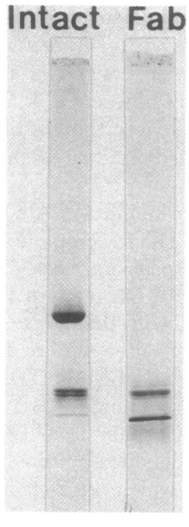

FIG. 1 Sodium dodecyl sulfate/5–11% polyacrylamide gel electrophoresis of purified 33D1 Ab before (Intact) and after (Fab) digestion with papain. The 33D1 Ig has a heavy chain with a molecular weight of 56,000, presumably an IgG, and two light chains, presumably one each from the rat spleen and mouse myeloma parents. The heavy chain is digested with papain.

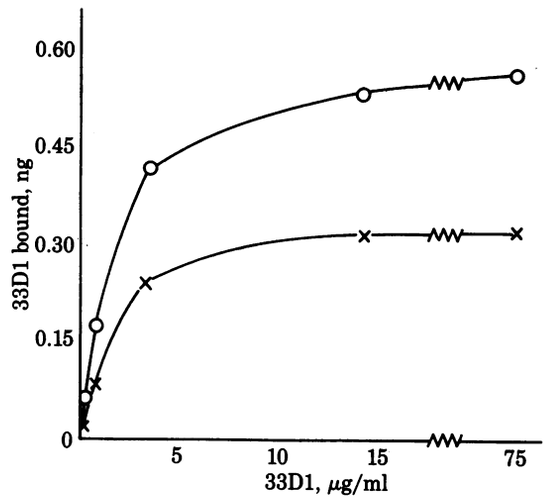

FIG. 2. Two experiments in which different concentrations of ^{3}H-33D1 and unlabeled 33D1 were added to 1.2 × 10^5 C3H (×) or 1.9 × 10^5 Swiss (○) spleen DC. Saturable binding was demonstrable.

Table 1. Specificity of ^{3}H-33D1 binding

Blocking antibody: Clone and specificity	^{3}H-33D1 bound, cpm	
	Exp. A	Exp. B
None	1707	NT
33D1, intact Ig, αDC	49	−40
33D1, Fab fragment	149	142
2E8, αI-A	1521	942
B21-2, αI-A	1638	903
B25-1, αH-2D	1660	921
2.4G2, αFcγ	1633	882
2.6, αDC + other cells	NT	981
2.D2C, αDC + other cells	NT	934

Spleen LODAC (2 × 10^5) were prepared from (DBA × BALB/c) (Exp. A) and Swiss (Exp. B) mice. Cells were exposed to a group of unlabeled monoclonal antibodies whose specificities have been described (4) for 60 min on ice. Then ^{3}H-33D1 was added at a final concentration of 3.5 μg/ml for another 60 min. Binding is expressed as cpm bound with cells minus cpm bound without cells (≈300 cpm) for duplicate cultures. In Exp. B, all cultures were supplemented with saturating levels of unlabeled 2.4G2 in addition to the other Ab. NT, not tested; α, anti-.

Table 2. ^{3}H-33D1 binding to lymphoid DC and MΦ

Test population (2×10^5 cells)	^{3}H-Labeled Ab bound, cpm			
	Exp. A (Swiss)		Exp. B (C3H)	
	33D1 (αDC)	2E8 (αI-A)	33D1 (αDC)	2E8 (αI-A)
No cells	5	−12	5	−6
Spleen DC	1626	3657	1283	1527
Node DC	1640	3164	1189	1695
Spleen MΦ	75	520	55	958
Spleen MΦ*	75	230	−11	730
Node MΦ	240	1570	−8	216

MΦ were purified from LODAC (7) or from high-density cells (*) (4) as described. In both techniques the MΦ were exposed to the same albumin columns used to purify DC. ^{3}H-Labeled Ab bound refers to cpm binding blocked by a 30-fold excess of unlabeled Ab; this is similar to cpm bound with cells minus cpm bound without cells.

RESULTS

To select for DC-specific Ab, hybridoma culture supernatants were screened against LODAC, the J774 MΦ cell line, and phytohemagglutinin-induced lymphoblasts, using an ^{125}I-labeled F(ab')$_2$ anti-Ig binding assay. Supernatants that selectively bound to LODAC were then tested for cytolytic activity in the presence of rabbit C. 33D1 was the only supernatant that was cytotoxic, killing 30–80% of cultured LODAC from all mouse strains (see *Materials and Methods*). Therefore the cells in this culture were cloned on soft agar and specificity for DC was established with quantitative binding, autoradiographic, and cytotoxicity assays.

Quantitative Binding Studies. Binding studies with radiolabeled primary Ab provide a sensitive means of quantitating specific antigens on small numbers of test cells (4). 33D1 binding to spleen DC saturated at 15 μg/ml, corresponding to 1.4×10^4 binding sites per DC (Fig. 2). By Scatchard analysis, the association constant was $\approx 4 \times 10^{-7}$ M^{-1}. The specificity of binding to DC was tested by blocking with unlabeled monoclonal Ab (Table 1). Both intact Ab and Fab fragments of 33D1 inhibited binding. A number of other monoclonal antibodies that react with DC, including anti-I-A reagents, did not compete with ^{3}H-33D1. Thus ^{3}H-33D1 binding to DC is saturable, is not Fc mediated, and identifies a novel DC determinant. All subsequent studies on the tissue distribution of 33D1 were performed with a dose of 3.5 μg/ml (60% saturation), because higher concentrations produced high levels of dish binding and were prohibitively expensive.

Table 3. ^{3}H-33D1 binding to mouse leukocytes

Test population	^{3}H-Labeled Ab bound, cpm	
	33D1 (αDC)	2E8 (αI-A)
Adherent to plastic		
No cells	7	4
Peritoneal MΦ, 3×10^5	−87	486
Blood monocytes, 3×10^5	13	102
Spleen MΦ, 3×10^5	186	363
Attached to poly(L-Lys)		
No cells	−15	20
Spleen DC, 3×10^5	3313	3905
Spleen, 6×10^6	684	2186
Node, 6×10^6	185	2981
Thymus, 6×10^6	31	540
Marrow, 6×10^6	133	490
Nonadherent peritoneal, 4×10^6	43	2269
Nonadherent blood, 4×10^6	5	1232
Blood platelets	14	244

Table 4. Enrichment of ^{3}H-33D1 binding after flotation and adherence

Test population	^{3}H-Labeled monoclonal Ab bound, cpm			
	Spleen		Lymph node	
	33D1 (αDC)	2E8 (αI-A)	33D1 (αDC)	2E8 (αI-A)
Unfractionated, 6×10^6	255	3039	240	1618
High-density, 6×10^6	86	2060	96	1338
LODAC, 3×10^5	1220	1606	2123	1699
Low-density nonadherent, 4×10^6	623	3026	244	2669

Spleen and lymph node from Swiss mice were separated into three subpopulations on the basis of buoyant density and plastic adherence (2, 3). Data are expressed as cpm blocked with a 20-fold excess of specific unlabeled Ab. The low-density fraction represented 5% of total spleen and 12% of total lymph node.

Binding of ^{3}H-33D1 was first tested on DC and MΦ from several organs. DC from spleen and lymph node bound similar levels of Ab (Table 2). MΦ from spleen, lymph node, peritoneal cavity, and blood (Tables 2 and 3) showed little or no binding, even when larger numbers of cells (2×10^6 spleen or peritoneal MΦ) were tested and even when MΦ expressed considerable levels of Ia Ag. In some experiments, low levels of ^{3}H-33D1 (about 1000 molecules per cell) bound to MΦ-rich preparations. This could be attributed to small numbers of DC contaminating these populations, to Fc-mediated binding (i.e., binding not inhibitable by 33D1 Fab), or to both. Three MΦ cell lines—J774, P388D1, and Ra 264.7—also lacked the 33D1 Ag (not shown).

Cells from six different tissues were screened next. Sensitivity was enhanced by using large numbers of test cells ($4–6 \times 10^6$). Specific ^{3}H-33D1 binding was detectable in spleen and corresponded to a level of 1 DC per 100–300 splenocytes. Binding above background was noted in lymph node, but was weak or absent in suspensions from thymus, peritoneal cavity, blood, and bone marrow (Table 3). Binding of ^{3}H-labeled 2E8, an anti-Ia reagent used as a positive control, was detectable in all preparations and did not correlate with the level of 33D1 Ag.

Spleen and lymph nodes were then fractionated by buoyant density and plastic adherences to prepare populations enriched in or depleted of DC by cytologic (3) and functional (6) assays. ^{3}H-33D1 binding correlated with DC content (Table 4). Specific binding was enriched 8- to 10-fold in the DC-rich low-density fraction. Of the binding in the low density fraction, 65–90% was in the adherent component. The latter represents less than 10% of the low-density cells and usually contains 75% or more of the DC.

Spleen DC, MΦ, and lymphocytes were tested after 4 days of culture. The level of 33D1 binding per DC fell approximately 50% (Table 5). MΦ and lymphocytes did not acquire the Ag

Table 5. Expression of 33D1 at various times in culture

Time in culture, hr	33D1 bound per 10^5 DC, ng
1–3	0.215 ± 0.050 (3)
18–26	0.164 ± 0.033 (9)
80–90	0.101 ± 0.026 (3)

Spleen LODAC were cultured for 1–90 hr and the percent DC was monitored by cytologic criteria (1, 2). Binding of 33D1 is expressed as ng of ^{3}H-33D1 bound (specific activity $\approx 4 \times 10^6$ cpm/ng), mean ± SD, with number of experiments in parentheses.

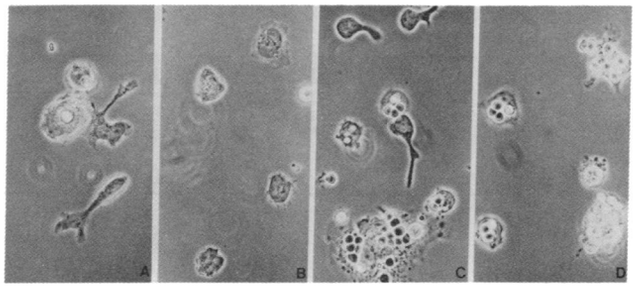

FIG. 3. Phase-contrast micrographs of lymph node LODAC obtained on albumin columns. Equal volumes of cells (10^7 per ml), Ab, and C were mixed and cultured for 1 hr at 37°C. Viable adherent cells attached firmly, nonadherent cells were removed, some of the preparations were fixed in 1.25% glutaraldehyde (*A* and *B*), and others were challenged with EA for 1 hr prior to fixation (*C* and *D*). (*A*) Adherent cells exposed to C without Ab. The field contains two typical DC, with many phase-dense mitochondria, and two ruffled round MΦ. (×340.) (*B*) Adherent cells prepared in the presence of 33D1 + C. DC have been killed but four typical MΦ can be seen. Cell counts of the entire coverslip show that DC numbers are reduced 80–90% while MΦ numbers are unchanged after exposure to 33D1 + C. (×280.) (*C*) Same as *A*, but fed EA. Four MΦ in the field have bound and interiorized EA (ingested EA are dark, and attached EA are light or refractile), while the three DC have not. (×280.) (*D*) Same as *B*, but challenged with EA. 33D1 kills DC, leaving an adherent population consisting primarily of MΦ that bind and phagocytose EA. (×280.)

(not shown). We conclude that 33D1 binding is specific for DC and can be used to monitor DC content in complex leukocyte mixtures.

Autoradiography. Autoradiograms were prepared from selected cell populations to visualize the 33D1 Ag at the single-cell level. Fresh and cultured spleen LODAC were 30–70% labeled, while whole spleen adherent cells were 5–15% labeled. Labeled cells were infrequent (<1%) in unfractionated spleen and lymph node and in purified spleen MΦ.

Cytotoxicity Assay. The next series of experiments established that DC were selectively killed by 33D1 plus C. LODAC were prepared from spleen and lymph node and exposed to C, 33D1 Ab, or both Ab and C. The latter killed 80–90% of the cells with the cytologic features of DC (compare Fig. 3 *A* and *B*). MΦ were not killed and continued to bind and phagocytose EA (compare Fig. 3 *C* and *D*). Specificity was further investigated by separating 1-day-cultured LODAC into DC- and MΦ-rich fractions by EA rosetting (2). Of the DC-enriched, EA^- fraction, 80–90% from both spleen and node were killed (Table 6). Only 0–10% of the MΦ-rich, EA^+ fraction were killed, and this low level could be attributed to DC, which are known to contaminate the EA^+ fraction (2). 33D1-mediated killing was not detectable either in cultured spleen or lymph node (Table 6) or in fresh unfractionated suspensions from spleen, thymus, peritoneal cavity, blood, marrow (Table 7), and lymph node (not shown). Controls with anti-Ig, anti-Thy-1, and anti-Ia produced appropriate levels of killing in all test populations (Tables 6 and 7).

Table 7. 33D1 + C fails to kill most mouse leukocytes

Ab (clone)	Cells killed, % Spl.	Thy.	PC	Blood	BM
Exp. A (C3H)					
None	4	4	4	3	3
αDC (33D1)	4	2	5	5	2
αI-A^k (10-2.16)	62	11	49	12	2
αI-$A^{b,d}$ (B21-2)	8	3	5	5	3
αThy-1 (B5-3)	39	100	36	63	3
αIg serum	56	5	11	11	4
Exp. B (B6 × D2)					
None	1	9	8	6	9
αDC (33D1)	4	8	7	4	10
αI-A^k (10-2.16)	4	9	7	4	11
αI-$A^{b,d}$ (B21-2)	50	16	25	19	12
αThy-1 (B5-3)	32	100	39	54	14
αIg serum	54	8	8	17	12

Cytotoxicity assays were run on leukocytes from spleen (Spl.), thymus (Thy.), peritoneal cavity (PC), blood, and bone marrow (BM). Antibody alone produced no killing above background.

Quantitative binding and autoradiographic assays were designed to determine whether 33D1 + C killed Ia^+ or Ia^- MΦ. After exposure to Ab + C, MΦ content was monitored with ^{125}I-labeled 1.21J (which binds to the mac-1 Ag on MΦ but not DC) and Ia was quantitated with ^{125}I-labeled B21-2 (anti I-$A^{b,d}$). Treatment with 33D1 + C produced no change in the level of either 1.21J or B21-2 binding in purified MΦ from blood, peritoneal cavity, or cultured spleen (Table 8). By autoradiography, the percentage of Ia-bearing cells was also unchanged (Table 8). In contrast, 33D1 + C treatment of fresh spleen LODAC, which contain substantial numbers of DC, decreased the amount of Ia but not 1.21J (mac-1) on the monolayer. This result indicates that the Ia-rich 1.21J-poor DC had been selectively removed. By autoradiography the percentage of $1.21J^+$ cells increased and $B21\text{-}2^+$ cells decreased after removal of most DC (Table 8). These cytotoxicity studies show that 33D1 kills DC but not other leukocyte, including Ia^+ and Ia^- MΦ.

Table 6. 33D1 specifically kills DC in cultured spleen and lymph node

Ab + C treatment	Cells killed in spleen, % EA^- (DC-rich)	EA^+ (MΦ-rich)	High-density (lymphocyte-rich)	Cells killed in lymph node, % EA^- (DC-rich)	EA^+ (MΦ-rich)	High-density (lymphocyte-rich)
No Ab	2	10	7	5	14	9
αDC (33D1)						
33 μg/ml	83	17	9	84	13	10
3.3 μg/ml	88	18	7	80	15	11
0.33 μg/ml	54	12	8	23	13	9
αThy-1 (B5-3)	2	8	28	6	12	55
αI-A (B21-2)	96	65	62	88	50	45
αIg	3	6	58	3	12	38

LODAC were prepared from spleen and lymph node, cultured overnight, and separated into DC- and MΦ-rich components after rosetting with EA. Cultured high-density cells were used as a lymphocyte-rich suspension. Cytotoxicity assays were carried out with monoclonal antibodies (except for anti-Ig) and agarose-adsorbed rabbit C. Killing did not occur when C was omitted.

Table 8. 33D1 + C does not kill Ia^- or Ia^+ MΦ

Adherent cells	No. of MΦ, cpm ^{125}I-1.21J (% 1.21J$^+$)		No. of Ia^+ cells, cpm ^{125}I-B21-2 (% B21-2$^+$)	
	No Ab/C	33D1/C	No Ab/C	33D1/C
Exp. A $(D_2 \times C)F_1$				
Cultured spleen LODAC (MΦ)	8,428 (97)	8,190 (98)	3,392 (26)	4409 (32)
Peritoneal cavity (MΦ)	19,624 (99)	19,213 (100)	458 (13)	446 (19)
Blood mononuclears (monocytes)	9,726 (94)	10,669 (96)	568 (12)	524 (9)
Fresh spleen LODAC (MΦ and DC)	4,198 (73)	4,113 (92)	13,171 (70)	5871 *(31)
Exp. B (C3H.OH)				
Cultured spleen LODAC (MΦ)	7,802 (98)	7,730 (98)	8,162 (58)	7402 (65)
Peritoneal cavity (MΦ)	13,287 (98)	14,574 (98)	3,732 (42)	3359 (38)
Blood mononuclears (monocytes)	3,722 (96)	3,427 (95)	1,737 (33)	1720 (30)
Fresh spleen LODAC (MΦ and DC)	3,202 (70)	2,570 (95)	8,100 (78)	2913 *(52)

Four populations of leukocytes ($1.5–3.0 \times 10^7$ per ml) were mixed with equal volumes of rabbit C with or without 33D1 Ab. The suspension (75 μl) was applied to 12-mm coverslips in triplicate for 1 hr at 37°C, allowing viable MΦ and DC to adhere firmly. Killing with 33D1 *during* rather than *after* adherence is preferable; there is less cell debris on the coverslip and less toxicity to MΦ in the no Ab/C control. Nonadherent cells were removed, and the adherent cells ($0.3–1.5 \times 10^5$) were exposed to saturating levels of ^{125}I-labeled monoclonal Ab on ice. 1.21J binds to the mac-1 Ag on MΦ but not DC (4) whereas B21-1 recognizes an $Ia^{b,d}$ determinant. After binding of ^{125}I-labeled Ab_1, the radioactivities on the coverslips were determined and mean cpm minus cpm without cells (≈200 cpm) was measured. Standard deviations were 10–20% for most triplicates. Statistically significant differences between no Ab and 33D1 (*t* test) are shown with an *. After bound cpm had been measured, the coverslips were processed for autoradiography. The percentage of labeled cells (>10 grains; 500 cells counted) is shown in parentheses. A positive control showed a fall in ^{125}I-labeled 1.21J binding after MΦ killing with B21-2 + C or with another cytotoxic Ab.

DISCUSSION

Clone 33D1 recognizes a DC-specific surface Ag. Cytotoxicity assays, quantitative binding studies, and autoradiography identified the Ag on most DC from spleen and lymph node. The number of 33D1 Ag copies (≈14,000) per DC is small; nevertheless, the assays used seem sufficiently sensitive to conclude that the Ag is not present on either Ia^+ or Ia^- mononuclear phagocytes (tissue MΦ or blood monocytes), lymphocytes, granulocytes, platelets, or erythroid cells.

The 33D1 Ag provides an independent marker that distinguishes spleen and lymph node DC from other leukocytes, including Ia-bearing cells. It is likely that DC represent a separate leukocyte lineage because their distinctive cytologic, functional, and surface properties, including expression of 33D1, are stable *in vitro*. The distribution of 33D1-bearing cells closely parallels the distribution of DC previously identified by cytologic (1, 3) and functional (6) criteria. In spleen and lymph node, this important cell type occurs at a very low frequency, less than 1% of the total cell suspension. Preparations from thymus, bone marrow, peritoneal cavity, and blood have even fewer DC. Expression of 33D1 also parallels DC content when various enrichment procedures are applied to spleen and lymph node. Both DC and 33D1 binding are enriched after adherence to glass or plastic, flotation on dense albumin columns, and removal of MΦ by EA rosetting and readherence. Given the specificity of 33D1, it is now appropriate to use the Ab to monitor the contribution of DC in functional systems and to outline the distribution of DC *in vivo*.

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