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ACCESSORY CELL–T LYMPHOCYTE INTERACTIONS.
Antigen-Dependent and -Independent Clustering

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T lymphocytes are stimulated when antigen is administered together with other cells, termed accessory or antigen presenting cells (APC). Unfortunately, the terms accessory cell and APC are broadly defined, and are being used to denote any cell that elicits a response in any population of responding T cells. If instead one compares the biological effects of specific types of APC, a salient feature is the strong stimulatory capacity of dendritic cells (DC) relative to other cell types during primary immune responses. This is evident in both culture systems in which antigen-specific responses can be generated, the MLR and the antibody response to T-dependent antigens (1–3). Other populations, such as macrophages and B lymphocytes, seem to exert their presenting function on lymphocytes that have already been activated by antigen in concert with DC (4–6). For example, primed T lymphoblasts bind to and deliver help to antibody-forming B cells in the apparent absence of DC, whereas the generation of primary and memory antibody responses requires DC (2, 5).

In trying to understand the special stimulatory properties of DC, we were faced with the fact that the antigen presentation step, which literally refers to the recognition of antigen in association with APC, is not assessed directly, as in binding assays between T cells and APC. Instead one measures some step distal to presentation, typically the accumulation of IL-2 in the medium or T cell proliferation, both of which occur after prolonged culture (>16 h) at 37°C. We recently encountered two situations in which T cells clustered to APC in short-term assays. The key was to use lymphocytes that were primed to Ia alloantigens in vitro. One type of binding was strictly antigen dependent, and involved the aggregation of T blasts with allogeneic B cells at 4°C (4). The other seemed to be antigen independent, since alloreactive T cells would aggregate with syngeneic as well as allogeneic DC, although only at 37°C (6). Similar antigen-independent clustering was reported for rat DC and primed T cells by Green and Jotte (7).

We now report detailed in vitro binding studies between sensitized T cells and different APC, including DC, macrophages, B cells, and Langerhans cells (LC). Using rapid-clustering assays, we find that most types of APC can interact with T cells in an antigen-dependent manner, although DC are the most effective. In contrast, it is only the DC that mediates an active form of antigen-independent clustering at 37°C.

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Abbreviations used in this paper: DC, dendritic cell; LC, Langerhans cell.
Materials and Methods

Preparation of T Lymphoblasts. T cells were nylon wool-nonadherent, spleen and lymph node cells from (C × D₂)F₁ (H-2d), BALB/c (H-2d), B10.A(3R) (H-2b/k/d), or B6.H-2k mice (The Trudeau Institute, Saranac Lake, NY; and The Rockefeller University). The populations were treated with mAb to Ia and Lyt-2 (4–6) to enrich for the Lyt-2⁻ subset that is restricted to class II MHC products. Lymphoblasts were sensitized to lectin (3 μg/ml Con A, Pharmacia Fine Chemicals, Piscataway, NJ), alloantigens, or soluble proteins (50 μg/ml of either KLH [Calbiochem-Behring, San Diego, CA] or OVA [Sigma Chemical Co., St. Louis, MO]). The procedure was as described (4–6), and the cell yields are summarized in Table I. Briefly, 5 × 10⁶ spleen DC were cultured with 5 × 10⁶ T cells in 1 ml medium (RPMI-1640 supplemented with 10% FCS 20 μg/ml gentamicin, and 5 × 10⁻⁶ M 2-ME) in 16-mm-diam culture wells. At 3 h or 2 d, clusters were isolated by velocity sedimentation in Percoll and cultured two more days, whereupon lymphoblasts were produced (Table I). Since the DC remained in clusters, the released blasts could be separated free of APC by another velocity sedimentation. The blasts were retreated with anti-Lyt-2 mAb and complement to remove residual Lyt-2⁺ cells. The distinct features of blasts relative to unprimed cells were their large size, rapid cell doubling (14–18 h) with human rIL-2 (Biogen, Cambridge, MA), and the capacity to specifically bind APC at 4°C (4, and see Results).

Antigen-presenting Cells. The APC populations we studied were: enriched spleen DC; resident and periodate-elicited peritoneal cells (25–60% macrophages); Sephadex G10–nonadherent spleen cells (~50% each of B and T lymphocytes); and epidermal cells. The latter were 12 h, low density, nonadherent suspensions (7–15% Ia⁺ LC), prepared by trypsin dissociation of ear epidermal sheets (8). In some experiments, the APC were cultured in 100 μM chloroquine (Sigma Chemical Co.) for 30 min at 37°C and washed twice before use.

T Cell Proliferative Assays. 3 × 10⁴ T blasts or 3 × 10⁵ unprimed T cells were stimulated with graded doses of spleen DC, with or without soluble proteins or lectins. At time points indicated in the results, 1 μCi of [³H]TdR was added and [³H]TdR uptake was measured 6–16 h later. Alternatively, 50 μl aliquots of the DC/T cell-conditioned medium was assayed for IL-2 activity on Con A-induced spleen T blasts.

Clustering Assays Between APC and T Lymphocytes. Graded doses of accessory cells were added on ice in 0.1 ml culture medium to 1.5-ml capacity, conical, polypropylene tubes (CT2075; Ulster Scientific, Highland NY). For experiments involving KLH- or OVA-primed cells, soluble protein antigens were then added, usually at 50 μg/ml. T cells, at 2 × 10⁶ cells/ml, were added last. The T cells were labeled beforehand with carboxyfluorescein diacetate (Sigma Chemical Co.), 5 μg/ml in culture medium for 5 min at room temperature. For Con A-induced blasts, the cells were washed twice with 10 mM α-
methyl mannoside. The mixtures of APC and T cells were centrifuged at 50 g at 4°C (500 rpm for 4 min in a Sorvall centrifuge with a 100836 swinging bucket rotor). For experiments at 37°C, the tubes were transferred to a water bath for 10 min and then returned to an ice bucket for counting. More prolonged incubation at 37°C resulted in the complete loss of T cell fluorescence. For experiments at 4°C, the tubes were left at least 10 min on ice before counting. More prolonged periods on ice did not alter the result, although the clusters could further aggregate with one another. To evaluate clustering, the cell pellets were slowly resuspended twice with a 100 μl pipetting device, and an aliquot was applied to a hemocytometer. The number of fluorescent cells that had not attached to nonfluorescent APC was counted under a low level of tungsten light in addition to UV. This permitted the simultaneous detection of nonfluorescent cells that were attached to the brightly fluorescent T lymphocytes. The difference between the number of single fluorescent T cells in control (no APC) and experimental (graded doses of APC) tubes was the number of clustered T cells. The fluorescent T cells showed little or no clustering in the absence of APC, and the average cell count was ~100, indicating full recovery of the added inoculum. In some cases (fresh epidermal cells and G10-nonadherent spleen cells), the APC population formed a few aggregates that lacked T cells. This did not interfere with the counting of fluorescent T cells or the formation of APC–T cell clusters (see Results).

Results

Since many of the APC–T aggregates were large (3–15 cells in diameter) and did not penetrate into the counting field, we required additional means to observe the APC–T cell interaction. To do so, we fixed the contents of each tube with an equal volume of 1.25% glutaraldehyde/PBS and transferred the cells to 16-mm wells for viewing by inverted phase contrast.

Our approach was to monitor the interaction of fluorescein-tagged T cells with APC after brief sedimentation (5 min) and association (≥10 min) steps. Two separable interactions were noted: antigen-dependent binding, which required specific antigen and APC of the appropriate MHC, and antigen-independent clustering, which did not need antigen or MHC restriction.

Stimulatory Requirements for T Blasts in Proliferation Assays. Assays of T cell growth and IL-2 release revealed the expected stimulatory requirements for each of the populations of T blasts that we had prepared. Blasts that had been primed to KLH or OVA were optimally restimulated with that specific protein and syngeneic DC (Fig. 1, A–D). The specificity was more striking with IL-2 release rather than growth assays, since the latter would measure nonspecific T blasts that responded to IL-2 released by other cells (Fig. 1, compare A and B with C and D). Blasts that were primed to alloantigen required stimulation with allogeneic DC (Fig. 1 E). Polyclonal lectin-induced blasts required lectin and DC that were either syngeneic or allogeneic (Fig. 1 F). The capacity of these enriched T blasts to interact with APC was then evaluated in binding assays.

Clustering of APC with Con A-induced T Cells. When DC and T blasts were brought together for just 5–10 min at 37°C, most of the cells formed large aggregates (Fig. 2, A–C; and Fig. 3). The clustering did not require addition of lectin, and 0.01 M α-methyl mannoside was present in the reaction mixture. MHC recognition also seemed unnecessary, since most of the cells in this polyclonal population clustered either syngeneic or allogeneic DC. Both Lyt-2− and L3T4+ blasts clustered efficiently with DC (Fig. 2, B and C), but no clustering was noted at 4°C (Fig. 3).

DC were the principal APC capable of clustering the Con A blasts at 37°C
FIGURE 1. Stimulatory requirements for lymphoblasts that have been sensitized in culture. Lyt-2-T cells from (C × D)F₁ mice were primed to 50 μg/ml KLH (A–D), 3 μg/ml Con A (F), or B6.H-2k alloantigens (F). Proliferation (A, B, E, F; 18-24h) and IL-2 release (C, D; 18 h) was then measured in cultures of 3 × 10⁴ blasts (A–D) or 2 × 10⁴ blasts (E, F). Where measured (A–E), the response to excess (10 U/ml) human rIL-2 is given at the top left of the graph. Background responses in the absence of DC are at the lower left. In the experiment with KLH-primed blasts (A–D), the stimuli were syngeneic (A, C) or allogeneic DC (B, D), plus the antigens that are indicated in the key. For lectin-induced blasts (E), the cells were stimulated with syngeneic (○, ●) or allogeneic (△, ▲) DC with (open symbols) or without (closed symbols) 1 μg/ml Con A. For alloreactive blasts (F), the H-2d anti-H-2k cells were restimulated with H-2k (○) or H-2d (●) DC. For the data on IL-2 release (C, D), the cpm value at 300 syngeneic DC plus KLH corresponds to 2 U/ml.

(Fig. 3, right). Peritoneal washout cells, which contained 25–40% macrophages but few DC, were at least 30 times less active than spleen DC. Splenic B and T lymphocytes were virtually inactive. Memory lymphocytes derived from the Lyt-2-T blasts clustered as well to DC as fresh Lyt-2-T blasts (not shown), but unprimed T cells clustered about 10 times less actively (Fig. 3, bottom right). We conclude that DC efficiently cluster T cells, particularly sensitized T cells, by an antigen-independent but temperature-dependent process.

Clustering of APC and Antigen-primed Helper T Blasts. H-2d, Lyt-2-T cells
were primed to H-2k allogeneic DC, or to H-2d DC plus KLH or OVA. Antigen-dependent clustering with T cells was then observed at 4°C. Alloreactive (anti-H-2k) T blasts and memory cells clustered H-2k but not H-2d DC (Fig. 2, D and E; and Fig. 4), while KLH- or OVA-reactive blasts required the specific protein (Figs. 5 and 6). The clustering of KLH- or OVA-primed blasts occurred with syngeneic but not allogeneic DC (Fig. 6), which is consistent with an MHC-restricted interaction. In contrast, at 37°C, APC/T cell clustering was antigen-
FIGURE 3. Antigen-independent clustering of APC with T cells. The APC were DC, peritoneal macrophages (Mφ) or splenic B cells (see key) and were added in graded doses to a constant number of T cells from (C × D2)F1 mice (H-2d). In the experiment on the left, allogeneic (B6.H-2k; top) and syngeneic [(C × D2)F1, bottom] DC cluster Lyt-2+ T blasts, but only at 37°C. In the experiment on the right, DC cluster T blasts (top) better than unstimulated Lyt-2+ T cells (bottom), and are much more active than macrophages or B lymphocytes.

FIGURE 4. Clustering of DC with H-2d anti-H-2k (alloreactive) T cells. DC were added in graded doses to (C × D2)F1 anti-B6.H-2k Lyt-2+ T blasts (left) or memory cells (right; prepared by resting T blasts for 3 d in the absence of alloantigen). At 37°C, both allogeneic (B6.H-2k) and syngeneic [(C × D2)F1] DC cluster, whereas at 4°C, B6.H-2k DC cluster in an antigen-specific fashion.
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Figure 5. Clustering of DC with KLH-primed T blasts. (C × D)F₁ Lyt-2− T blasts were mixed with graded doses of DC and no KLH, 1 μg/ml KLH, or 50 μg/ml KLH (see key). Note that the DC clustered with the T blasts in an antigen-independent manner at 37°C, and in an antigen-dependent manner at 4°C.

Figure 6. Clustering of DC with antigen-primed blasts is self-restricted and antigen-dependent. H-2d, Lyt-2− T blasts were primed to antigen and then clustered at 4°C with graded doses of DC from syngeneic or allogeneic (B6-H-2k) mice, with or without 50 μg/ml KLH or OVA (see key). Note that clustering of the KLH- or OVA-primed blasts is antigen-dependent and self-restricted. At 37°C, the clustering of the same cells (not shown) was antigen-independent and MHC-unrestricted.

independent and was as efficient as that observed with lectin-primed T cells. H-2d anti-H-2k blasts or memory cells clustered H-2d as well as H-2k DC (Fig. 4), and KLH-primed T blasts clustered in the absence of KLH (Fig. 5, left).

Other APC were then compared to DC. B cells formed clusters in an antigen-specific fashion, as described previously (4) (Fig. 7). B cell clustering was similar at 4 and 37°C, but the efficiency of B cells was some 10–20-fold less than DC
Figure 7. A comparison of DC with B cells in clustering alloreactive T cells in different states of activation. The APC were allogeneic DC or B cells (G10-nonadherent spleen cells; this population contains ~50% T cells that are not capable of clustering). Note that B cells cluster T blasts at 4 or 37°C but did not cluster unprimed or memory T cells at either temperature.

Figure 8. A comparison of DC with peritoneal macrophages in clustering alloreactive T blasts. At 4°C (left), allogeneic DC and sodium periodate-elicited peritoneal exudate cells (Mφ) cluster the alloreactive T blasts. At 37°C, the DC are >100 times more active than macrophages in antigen-independent clustering (top right).

(Fig. 7). No antigen-independent clustering was observed at 37°C (not shown). B cells only clustered freshly sensitized T blasts, but DC could cluster both blasts and memory cells (Fig. 7). Peritoneal cells also exhibited antigen-dependent but not -independent binding at both 4 and 37°C (Fig. 8). The macrophages, by virtue of their large size and cytoplasmic granules, were present in the clusters. However, peritoneal cells were 10 times less active than DC in the binding assay. The clusters induced by B cells and macrophages often had a rosette appearance with a central T blast. The DC clusters were larger and contained many T cells and interspersed DC (not shown).
Effects of Chloroquine. Treatment with lysosomotropic agents, like chloroquine, has been found to block presentation of soluble protein but not allogeneic Ia antigens (9). The addition of chloroquine to our binding assays at 4°C did not alter APC/T cell binding. However, a half-hour pretreatment at 37°C with 100 μM chloroquine markedly inhibited the capacity of APC to bind helper T blasts in an antigen-dependent manner. Recognition of soluble proteins and alloantigens at 4°C were both blocked by chloroquine pretreatment (Fig. 9). The same result was obtained using DC or macrophages as APC (not shown). Antigen-independent clustering at 37°C was unaffected by the drug (Fig. 9).

The chloroquine-pretreated DC were tested as stimulators of the primary and secondary MLR. Others (9) had noted that chloroquine does not block allogeneic stimulation, and we confirmed this for the primary MLR at concentrations of <100 μM (Fig. 10, right). However, stimulation of primed blasts was blocked by 90% if the DC had been preexposed to 100 μM chloroquine. Since responses to allogeneic Ia were blocked by chloroquine, it is possible that the drug can act
Figure 10. Effect of chloroquine pretreatment on the stimulation of unprimed T cells and T blasts in the primary and secondary MLR, respectively. (C × D)F₁ DC were treated with chloroquine for 30 min before use as stimulators against Lyt-2- B10.A (3R) T cells. Note that 100 μM chloroquine has no blocking effect on the primary MLR but shifts the dose response for secondary stimulation by a factor of 10.

Figure 11. Clustering capacity of epidermal cell suspensions. (C × D)F₁ anti-B6.H-2k T blasts were mixed at 4 or 37°C with syngeneic or allogeneic spleen DC; allogeneic, 12-h, low-density epidermal cells (13% IA⁺ LC); or syngeneic, 12-h, low-density epidermal cells (7% IA⁺ cells). The doses on the abscissa are IA⁺ cells, since the IA⁻ cells in the epidermal suspensions were incapable of clustering (not shown). Note that 12-h LC cluster with alloreactive blasts in an antigen-dependent fashion, but do not exhibit antigen-independent binding at 37°C.

Directly on IA molecules, and that this effect is reversible with long-term assays like the primary MLR.

Clustering of T Cells and Epidermal LC. Freshly isolated, IA-rich, epidermal LC are weak or inactive in inducing T cell proliferation in the MLR, and in oxidative mitogenesis (8). We asked if either antigen-dependent or -independent forms of clustering were defective in LC. At 4°C fresh epidermal cells bound to alloreactive T blasts in an antigen-dependent fashion (Fig. 11, left). The clustering likely involved the IA⁺ LC, which are the main IA⁺ cells in epidermis (8). IA⁺
epidermal cells have been isolated by cell sorting and do not cluster (Inaba and Steinman, manuscript in preparation), and the clustering capacity of epidermal cells was identical to spleen DC when the dose was expressed in Ia⁺ equivalents (Fig. 11). At 37°C, fresh epidermal cells did not form antigen-independent clusters. This deficiency could not be ascribed to the use of trypsin in LC dissociation, since 0.25% trypsin in PBS for 15 min did not affect the clustering capacity of spleen DC (not shown). Therefore, the lack of stimulating capacity for primary responses correlates with a deficit in antigen-independent binding rather than antigen-specific clustering.

Discussion

Rapid-binding Assays Between APC and T Lymphocytes. We have studied the direct interaction of APC and T lymphocytes to try to understand why different types of APC vary so markedly in their capacity to stimulate immune responses. The assay we used grew out of previous data on the primary MLR and antibody response to foreign red cells and hapten-carrier conjugates (4-6). We had noted that DC initiated the development of stable aggregates that contained most of the DC and responding T lymphocytes in the culture. Other APC, like B cells, could subsequently enter the clusters. To evaluate the cell–cell interaction more directly, we had to select populations that were highly enriched in antigen reactivity. This was accomplished using previously described (4-6) techniques in which primed lymphoblasts were derived from DC/T cell clusters. The blast populations contained 50-80% specific antigen-binding cells. The use of T cell clones were avoided for these first experiments, because of a concern that the cloning technique would select and/or alter the properties we wished to measure. Preliminary observations indicated that T cells maintained for additional days in IL-2 do not exhibit the same clustering efficiency as freshly sensitized lymphocytes. In addition, we wanted to study bulk oligoclonal populations rather than risk selection of a minor monoclonal population that would be atypical in terms of receptor affinity or efficacy of binding to APC.

The assay was a rapid one in which APC and fluorescent T cells were sedimented together, kept at 4 or 37°C for 10 min, and then gently resuspended before counting in a hemocytometer. In the case of DC (Fig. 2) and macrophages (not shown), it was evident that most of the added APC were clustering with the blasts. Therefore, it should prove feasible to monitor aggregation using fluorescent APC as well as fluorescent T cells.

With DC, a key variable in detecting antigen-dependent clustering was using a low temperature, since marked antigen-independent binding occurred at higher temperatures. At 4°C and at high DC/T ratios, only a small amount of apparently nonspecific clustering was observed. The latter may really represent the specific aggregation of contaminating T cells that recognize self MHC or self plus antigens in FCS. In the case of other types of APC, antigen-dependent clustering in suspension was evident at 4 or 37°C. Another important feature of the assay was to use T blasts that were freshly sensitized in culture. If the blasts were rested (i.e., memory cells), their clustering capacity with B cells was diminished. We are now comparing T blasts and memory cells further to determine whether
these differences can be attributed to variations in the number or mobility of T cell receptors for antigen plus MHC.

Others (10-12) have studied the interaction of T cells with adherent peritoneal exudate cells. At 37 °C, they noted antigen-dependent and -independent attachment of T cells, but only after incubations for >1 h. The T cells did not seem to bind to adherent macrophages, but rather to a central cell of uncertain lineage (10), possibly DC. Since antigen-specific T cells were not enriched beforehand, and since the level of antigen-independent clustering was very high, it was only feasible to monitor the efficacy of clustering by depletion of function from the nonattached fraction. Unfortunately ~80% of the applied cells, which were largely antigen-nonspecific, attached to the monolayers when extensive depletion of functional activity was obtained (12). We are concerned that the use of monolayers of exudate cells is not an optimal approach for binding assays. Our preference is to study binding in suspension. It is technically simpler; it allows one to study important classes of APC that are nonadherent, such as DC and B cells, and to add graded doses of APC; and it can identify two separate types of clustering that are predominantly antigen-dependent or -independent.

Antigen-dependent Clustering at 4°C. The most important finding from the experiments performed at 4°C was that all types of APC (DC, LC, macrophages, and B cells) could interact with T cells in an antigen-dependent fashion. This kind of binding is perhaps the most direct assay for antigen presentation that is currently available, although it does not directly monitor other events, such as antigen processing and production of IL-1. While DC were more efficient in presenting antigen in binding assays at 4°C, the difference between DC and other cells does not readily account for their enhanced immunostimulatory capacity. Thus, B cells and macrophages are weak inducers of the primary MLR and T-dependent antibody responses (1-3), yet these same APC can clearly interact with T cells in an antigen-dependent fashion, including stimulation of secondary proliferative responses (4-6).

The minimal mechanism underlying antigen-dependent clustering at 4°C is an interaction between antigen/MHC on the APC and the Ti receptor for antigen/MHC. The role of other accessory molecules is being evaluated. Our current data do not address the need for temperature-dependent antigen processing before the presentation step. We have not taken precautions to rid our protein preparations of denatured protein or peptide fragments that might be presented without further modification, nor have we selected antigens in which the epitopes that must be recognized by T cells are not available in the native protein. However, it is possible that the T cells we have primed in vitro can recognize native protein, since a relatively low concentration (1 μg/ml) produced detectable APC-T cell clustering at 4°C (Fig. 5).

The effects of chloroquine on the APC-T interaction were quite striking. It is usually assumed that the inhibitory effects of chloroquine on APC function are at the level of protein processing. However, we find that chloroquine blocks alloantigen presentation in short term assays, either clustering at 4°C or stimulation of primed blasts at 37°C (Figs. 9 and 10). Therefore, one must entertain the possibility that chloroquine acts directly on the presenting function of Ia molecules. There are instances in which chloroquine rapidly downregulates the
level of a specific plasma membrane constituent in the apparent absence of ligand. For example, chloroquine leads to the rapid ($t_{1/2}$, 6 min) sequestration of asialoglycoprotein receptors from the hepatocyte plasma membrane into endocytic vacuoles (13). Possibly, this drug removes from the cell surface a critical pool of Ia molecules or an associated molecule such as the chondroitin sulfate-like moiety described by Sant et al (14). Another perhaps important effect of chloroquine is that it retards the normal dissociation of invariant-chain molecules from newly synthesized Ia $\alpha/\beta$ heterodimers in B cell lines (15).

Previous studies (4, 5) have shown that B cells can cluster with primed T blasts during the development of antibody-secreting cells. However, B cells did not initiate clustering when the T cells were unprimed, or when IL-2 unresponsive memory lymphocytes were used. Therefore, we suspect that antigen-dependent clustering of T blasts has an important function in the effector limb of T-dependent responses, when sensitized T cells must release lymphokines and/or act directly on such APC as B cells and macrophages. In the case of clustered B cells, responsiveness to helper factors seemed to be induced in T cell aggregates (4).

**Antigen-independent Clustering at 37°C.** This form of APC–T cell binding was primarily seen with DC. B cells, macrophages, and fresh LC were virtually inactive, even though the same cells could cluster at 4°C in an antigen-dependent fashion. There is evidence that antigen-independent clustering does not involve Ia molecules on DC or T4 molecules on the primed T cell (7). Other moieties like the lymphocyte functional antigens have not been evaluated.

Antigen-independent clustering may underlie the immunostimulatory properties of DC, since this is the first mechanistic feature that so markedly distinguishes DC from other APC. Clustering could generate separate signals required for lymphocyte activation or provide a microenvironment that enhances the efficacy of antigen presentation and cytokine function. Supportive evidence comes from our preliminary data with epidermal cells. Freshly isolated LC lack stimulatory function in the primary MLR (8). This deficit is not readily attributed to defective presentation, since the LC present alloantigens in binding assays at 4°C (Fig. 11). We are now comparing highly enriched populations of fresh and cultured LC, and are finding that the latter develop the capacity to cluster in an antigen-independent way, and can stimulate primary responses in culture.

**Summary**

Previous work documented the capacity of dendritic cells (DC) to stimulate primary immune responses and to physically cluster with the responding lymphocytes. Rapid cell-cell aggregation assays were used here to study the interaction of DC and other types of APC with T lymphocytes. Graded doses of APC were sedimented with T cells that had been primed to alloantigens, soluble proteins, or lectin, and then labeled with carboxyfluorescein diacetate. The number of clustered T cells was measured after 10 min at 4 or 37°C.

At 4°C, binding was antigen-dependent and included $\geq$50% of the added T cells. Clustering was mediated by all types of APC tested, including DC, macrophages, B lymphocytes, and fresh Langerhans cells, although DC were the most effective. Specificity was evident in the findings that alloreactive T lymphoblasts
bound to allogeneic but not syngeneic APC; KLH- and OVA-reactive T cells bound to syngeneic APC in the presence of specific protein; and Con A blasts needed lectin to cluster.

A 30 min pretreatment with chloroquine, a drug known to inhibit APC activity, markedly blocked the specific binding of alloreactive and protein-specific T blasts at 4°C. Since Lyt-2+ alloreactive blasts should specifically recognize Ia, presentation of Ia seems to be altered by chloroquine.

Binding assays at 37°C gave similar results to those performed at 4°C, with one exception. When DC were used as APC, striking antigen-independent clustering occurred. DC could efficiently cluster primed T cells in the absence of alloantigen, soluble protein, or lectin. We suggest that antigen-independent binding contributes to the distinctive capacity of DC to prime T cells in the afferent limb of the immune response, whereas antigen-dependent binding between other APC and sensitized lymphocytes is critical in the efferent limb.

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