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# T-cell activation by dendritic cells: CD18-dependent clustering is not sufficient for mitogenesis

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## SUMMARY

The physical association of dendritic cells and T lymphocytes in clusters is required for primary immune responses. We have used oxidative mitogenesis as a model to examine the requirements for accessory cell–T-cell clustering and T-cell activation; in this polyclonal response, periodate-treated T cells cluster with dendritic cells (DC) and proliferate. Here we observed firstly that macrophages (M$\phi$) but not B cells or B blasts could also cluster with periodate-treated T cells, but they did not stimulate proliferation. Thus, clustering with a cell that can express MHC class II (Ia) molecules and synthesize interleukin-1 (IL-1) is not sufficient for mitogenesis, and Ia expression is not sufficient for clustering. Secondly, that proliferation in oxidative mitogenesis and the allogeneic mixed leucocyte reaction (MLR) was inhibited by CD18, CD4 and CD8 antibodies but not, with one exception, by others of the panel tested. Thirdly, using rapid cluster assays we showed that DC and M$\phi$ formed temperature-dependent clusters with syngeneic and allogeneic periodate-treated T cells. Clustering was inhibited by CD18 antibodies, probably at the level of the T cell but not the accessory cell, and this may be a general feature of such inhibition. However, CD4 and/or CD8 antibodies did not affect clustering, showing that these molecules are involved in subsequent stages of T-cell activation. Since clustering of DC and M$\phi$ with periodate-treated T cells requires CD18, but only the former leads to proliferation, we conclude that CD18-dependent clustering is not sufficient for mitogenesis.

## INTRODUCTION

Dendritic cells (DC) are uniquely required as accessory cells for primary T-dependent immune responses (reviewed by Austyn, 1987). These responses occur in cell aggregates or clusters, but little is known of the molecules that are involved in the clustering of DC and T cells. In previous studies we have used oxidative mitogenesis as a model to examine the mechanism of action of DC. In this system T cells that have been treated with sodium periodate proliferate in the presence of DC in a rapid and polyclonal response. We have shown that DC are the principal accessory cells for this response and that they stimulate T cells to release and become responsive to IL-2, which then causes proliferation of the responsive T cells (Austyn *et al.*, 1983). Furthermore, clustering with DC precedes and is essential for this T-cell activation (Austyn, Weinstein & Steinman, 1988). In this study, we show that M$\phi$ but not B cells or blasts can also cluster with periodate-treated T cells, but that mitogenesis is not induced. This has allowed us to compare some of the molecular requirements for T-cell clustering with DC, which eventually leads to proliferation, to that with M$\phi$, which does not.

Abbreviations: DC, dendritic cells; IL, interleukin; MHC, major histocompatibility complex; MLR, mixed leucocyte reaction; M$\phi$ macrophage.

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## MATERIALS AND METHODS

*Mice*

C57BL/10 (H-2b) and BALB/c (H-2d) mice were from Olac Ltd, Bicester, Oxon. Mice were of both sexes and 6–12 weeks of age.

*Culture media*

The medium used throughout was RPMI-1640 supplemented with 5–10% heat-inactivated fetal calf serum, 100 U/ml penicillin and streptomycin, and 2-mercaptoethanol ($5 \times 10^{-5}$ M; Bio-Rad Laboratories, Richmond, CA). All except the latter were from Flow Laboratories (Rickmansworth, Herts). For experiments in Figs 1–3, indomethacin (1 $\mu$g/ml; Sigma, St Louis, MO) was added. Cells were washed with RPMI-1640 or, where indicated, with PD, which is phosphate-buffered saline without calcium and magnesium (Flow Labs).

*Monoclonal antibodies*

Antibodies used in this study were as follows; those preceded by TIB were from the American Type Culture Collection: Rat

IgG2b: 2.4G2 anti-Fc receptor (FcR; Unkeless, 1979); 33D1 anti-DC (Nussenzweig *et al.*, 1982); TIB 213, clone FD441.8, anti-lymphocyte function-associated antigen 1 (LFA1 = CD18); B5-5 anti-Thy-1.2 (Steinman *et al.*, 1980); TIB 207, clone GK1.5, anti-L3T4 (CD4); F4/80 anti-M$\phi$ (Austyn & Gordon, 1981). Rat IgG2a: TIB 122, clone M1/9.3.4, anti-leucocyte common antigen (LCA); TIB 104, clone 53-7.313, anti-Ly 1; TIB 105, clone 53-6.72, anti-Ly 2 (CD8); KJ16-133 anti-T-cell receptor (Roehm *et al.*, 1984).

*Cells*

*Dendritic cells and T lymphocytes.* These were prepared as described elsewhere (Austyn *et al.*, 1983; Austyn *et al.*, 1988).

*Resident peritoneal macrophages.* Resident peritoneal cells (RPC) were obtained by peritoneal lavage using PD. For the experiment in Fig. 1, $5 \times 10^5$ cells were cultured in 16-mm wells (Linbro 76-063-05; Flow Labs) for 2–3 hr. They were then washed extensively to remove non-adherent cells, before immediate addition of the cell types shown. For the experiment in Fig. 2, RPC were incubated at $1 \times 10^7$/100-mm petri-dishes (Falcon 1029; Becton-Dickinson Labware, Oxnard, CA) for 2–3 hr before washing, to leave adherent cells which were cultured 18 hr overnight. The following day, the cells that had become, or were readily, detached were replated for 2 hr before harvesting and used as 'non-adherent peritoneal cells'. The firmly adherent cells were removed by a protocol for which we are indebted to Dr Paul Crocker, Sir William Dunn School of Pathology, University of Oxford: the cells were rinsed in cold PD to remove serum and incubated on ice for approximately 5 min in PD plus 5 mM glucose, followed by vigorous pipetting. The released 'macrophages' were routinely >98% viable.

*B cells and blasts.* For B cells, spleen cells were incubated on columns of Sephadex G10 (Sigma), and the non-adherent population was treated with phosphate-buffered ammonium chloride to lyse erythrocytes (Smith *et al.*, 1986). To prepare lymphoblasts, B cells were cultured at $2 \times 10^6$/ml in lipopolysaccharide (10 $\mu$g/ml; a gift of Dr Alain Townsend, Nuffield Department of Medicine, University of Oxford) for 3 days.

*Cluster assays*

These were a modification of those described by others (Green & Jotte, 1985; Inaba & Steinman, 1986). T cells were resuspended to approximately $5–10 \times 10^6$/ml in culture medium as above, but containing 2% fetal calf serum. They were incubated with the DNA-intercalating fluorochrome Hoescht 33342 (6 $\mu$g/ml; Sigma) for 15 min at 37° and washed twice (Brenan & Parish, 1984). Unlabelled DC and labelled T cells were resuspended in ice-cold culture medium containing 10 $\mu$g/ml DNase (Sigma). T cells were distributed into 1·5 ml microcentrifuge tubes (LW2075; Alpha Labs, Eastleigh, Hamps) on ice, with or without antibodies, at a final density of $10^6$/ml, with DC at the indicated ratio, in a total volume of 100–200 $\mu$l. For preincubation, the cells were left on ice for 45–60 min. The cells were then sedimented at 500 ***g*** for 4 min at 4°, and either transferred to a 37° water bath for 10 min and then placed on ice, or placed on ice directly, where they were left until counting. They were gently resuspended twice, placed in a haemocytometer and examined under a UV microscope. Blue-fluorescing T cells that were not associated with unlabelled DC in clusters were counted, and the number of clustered cells obtained by difference; results are the means of four estimations.

## RESULTS

### Macrophages can cluster with periodate-modified T cells, but do not induce proliferation

Previous experiments have shown that Ia-positive splenic M$\phi$ are weak or inactive at stimulating oxidative mitogenesis (Austyn *et al.*, 1983). However, M$\phi$ could cluster with periodate-modified T cells. This phenomenon was most readily demonstrated with adherent peritoneal M$\phi$ (Fig. 1b), to which the T cells rapidly attached. When non-adherent cells were removed at 3 hr, every M$\phi$ was clustered with lymphocytes, and no clusters were seen in the non-adherent cells (compare Fig. 1c and d). By 24 hr, all of the T cells that had clustered with M$\phi$ had detached (Fig. 1g), and this was also seen in cultures that were not disturbed at 3 hr (not shown). In contrast, when DC were also added to the cultures, periodate-modified T cells seemed to cluster preferentially with DC: at 3 hr none were in contact with adherent M$\phi$ (Fig. 1e). Instead, clusters were present in the non-adherent fraction (Fig. 1f), and these increased in size by 24 hr (Fig. 1h). No clusters were evident if accessory cells were not added (Fig. 1a), or if the T cells were not periodate treated (not shown).

Despite the ability of periodate-treated T cells to cluster initially with M$\phi$, no proliferation was induced unless DC were also added (Fig. 2). M$\phi$ actually suppressed responses to DC, even with indomethacin in the medium to reduce the inhibition caused by prostaglandins. In a number of experiments using unfractionated resident peritoneal cells or freshly isolated adherent M$\phi$, some mitogenesis was induced (not shown). However, the stimulatory activity was present in the cells that detached overnight (Fig. 2) and in which morphologically typical DC were seen (not shown). We conclude that physical association with M$\phi$, a cell that can express Ia and produce IL-1, is not sufficient to induce T-cell proliferation.

### B cells and B blasts are essentially unable to cluster with periodate-modified T cells, or to induce mitogenesis

We tested the ability of splenic B cells and LPS-stimulated B blasts to act as accessory cells for oxidative mitogenesis. Clusters did not form in microcultures containing B cells (not shown) and T-cell proliferation was not induced (Fig. 3). This was so whether or not the B cells were irradiated. Some small clusters developed with high doses of B blasts, but these were only evident after 8–12 hr of culture (not shown) and only a small amount of DNA synthesis subsequently occurred (Fig. 3). Thus, Ia expression is not sufficient for clustering.

### Proliferation in oxidative mitogenesis is inhibited by CD18, CD4 and CD8 antibodies

We examined a pnael of monoclonal antibodies for their inhibitory effects on oxidative mitogenesis and on the allogeneic MLR for comparison. One representative experiment (of 3–9 for each antibody) is shown in Table 1. Proliferation in both responses was inhibited by CD18 (LFA1), CD4 (L3T4) and CD8 (Ly 2) antibodies. It was abrogated by CD18 antibodies in

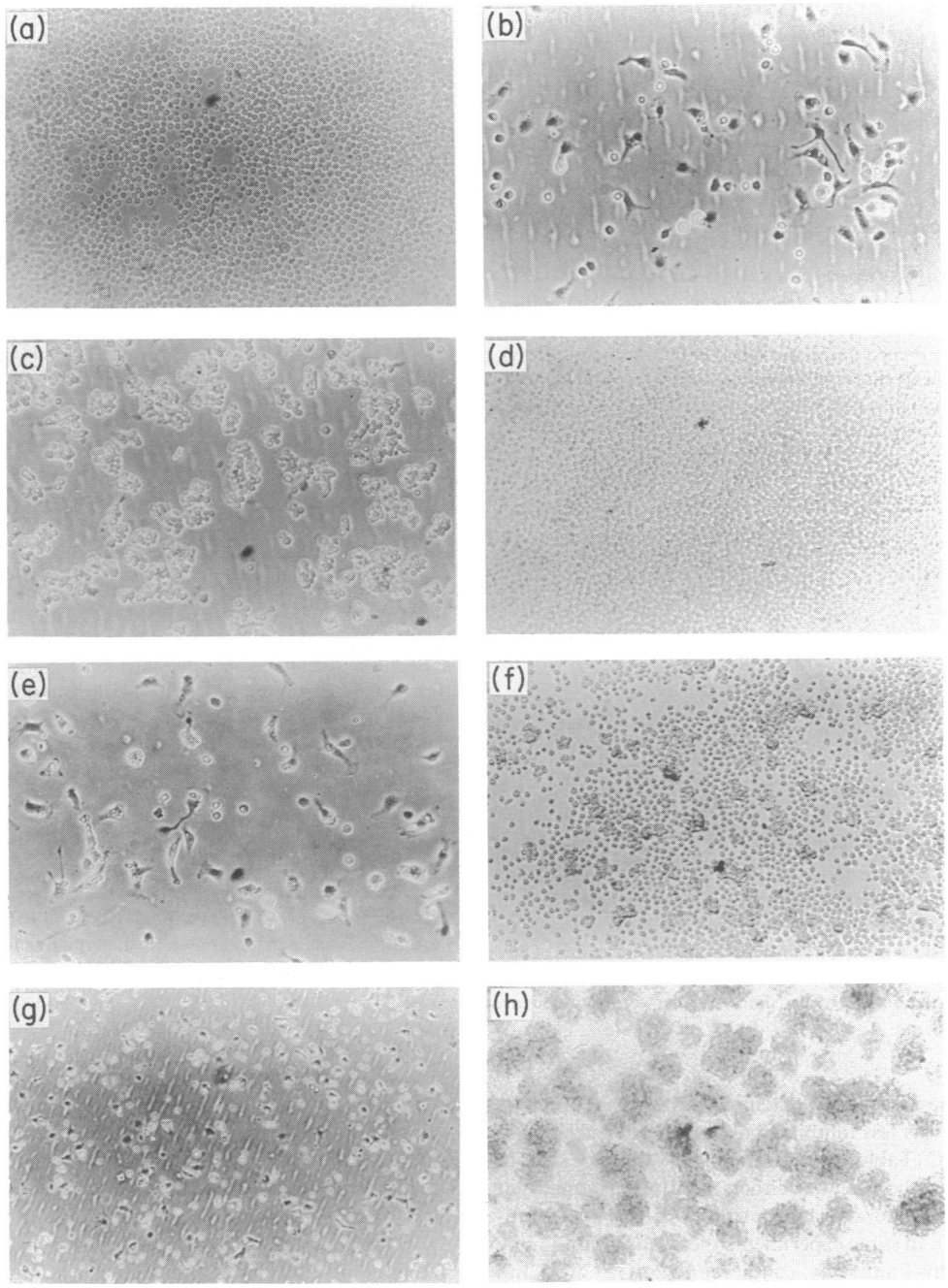


**Figure 1.** Periodate-modified T cells can cluster with peritoneal macrophages. Adherent monolayers of peritoneal M$\phi$ were prepared (estimated at $1-2 \times 10^5$/16-mm well) and $2 \times 10^6$ periodate-modified T cells were added, with or without $1 \times 10^5$ DC. After 3 hr culture the non-adherent cells were gently resuspended and transferred to new wells, and the remaining adherent cells were carefully washed in warm medium. Both populations were photographed at this stage (3-hr), or after they were returned to culture for a further 21 hr (24-hr) at which time the cells were not manipulated in any way. (a) 24-hr T cells alone ( $\times$ 13); (b) 3-hr M$\phi$ alone ( $\times$ 20); (c) 3-hr adherent cells from M$\phi$ + T cultures ( $\times$ 13), all M$\phi$ have formed clusters; (d) 3-hr non-adherent cells from M$\phi$ + T ( $\times$ 13), no clusters are in evidence in suspension; (e) 3-hr adherent cells from DC + M$\phi$ + T cultures ( $\times$ 13), clusters with M$\phi$ have not formed; (f) 3-hr non-adherent cells from DC + M$\phi$ + T ( $\times$ 13), clusters are seen; (g) 24-hr culture from (c) ( $\times$ 6·5), the initial clusters have spontaneously dissociated; (h) 24-hr culture from (f) ( $\times$ 6·5), the clusters have increased in size.

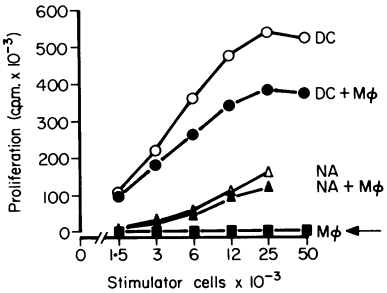


**Figure 2.** Adherent peritoneal macrophages do not induce proliferation of periodate-treated T cells. Periodate-modified T cells were cultured in microtitre wells with graded doses of irradiated DC, Mϕ (see arrow), non-adherent peritoneal cells (NA), or mixtures of them (equal numbers as indicated), and proliferation was measured. Similar results were obtained in a second experiment.

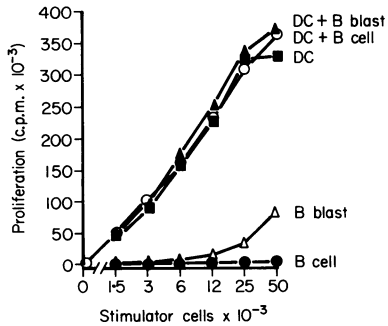


**Figure 3.** B cells and B blasts do not induce significant proliferation of periodate-modified T cells. Periodate-modified T cells were cultured in microtitre wells with graded doses of irradiated DC, B blasts, B cells, or mixtures of them, and subsequent proliferation was measured. Only results with unirradiated B cells are shown as these were indistinguishable from those with irradiated B cells. Similar results were obtained in a second experiment.

oxidative mitogenesis and was not overcome by higher numbers of DC, but there was less inhibition of the MLR, especially at higher doses of DC (Table 1). Inhibition in the presence of both CD4 and CD8 antibodies was either additive or somewhat synergistic relative to the subclass controls (anti-Thy-1 and anti-Ly 1, respectively). In some experiments there was inhibition by the IgG2b anti-Thy-1 antibody, compared to 2.4G2, 33D1 and F4/80 (Table 1).

We also examined the time-course of inhibition by CD18 and CD4 antibodies (Table 2; CD8 not tested). In oxidative mitogenesis there was a linear relationship between the time of addition of both antibodies and the degree of inhibition, which was zero at about 22–24 hr when DNA synthesis commenced. Neither antibody inhibited when added after this time. For the MLR both antibodies inhibited maximally when added early, but the relationship at later times was less clear.

### Dendritic cells and macrophages can form temperature-dependent clusters with syngeneic and allogeneic periodate-treated T cells

Rapid cluster assays were used to examine the early clustering of DC and Mϕ with periodate-treated T cells. Fluorescently labelled T cells were sedimented together with unlabelled DC or Mϕ and incubated for a short period of time (10 min). Labelled (T) cells that were not associated with unlabelled cells were counted, and the number of clustered cells was obtained by difference as it was difficult to count these directly.

Stable clusters formed when DC or Mϕ were incubated at 37° with syngeneic or allogeneic periodate-treated T cells (Table 3), but not with untreated T cells (not shown). About half of the T cells could cluster and a plateau of binding was seen at ratios of DC or Mϕ:T of about 1:1 (not shown) to 1:4 (Table 3). This was not increased during incubations of up to 60 min at 37° (not shown). Clustering did not occur at 4°, although there was a small amount of what appeared to be temperature-independent aggregation (Table 3). Thus, DC and Mϕ cluster with periodate-treated T cells in a temperature-dependent manner.

**Table 1.** Inhibition of proliferation in oxidative mitogenesis and the allogeneic MLR by monoclonal antibodies

| Antibody specificity* | Response (c.p.m. × $10^{-3}$) to graded doses (× $10^{-3}$) of DC in: Oxidative mitogenesis† 25 | 8 | 2·5 | Allogeneic MLR‡ 25 | 8 | 2·5 |
|---|---|---|---|---|---|---|
| FcR | 313·9 | 140·0 | 71·5 | 299·0 | 212·7 | 91·8 |
| 33D1 | 290·2 | 174·4 | 68·9 | 255·7 | 197·2 | 93·2 |
| CD18 | 14·9 | 5·2 | 2·0 | 127·2 | 62·2 | 12·9 |
| Thy-1 | 170·8 | 101·3 | 47·9 | 267·9 | 179·7 | 86·3 |
| CD4 | 100·2 | 52·6 | 21·2 | 119·0 | 83·1 | 19·1 |
| LCA | 272·0 | 153·0 | 84·4 | 338·6 | 237·7 | 146·6 |
| Ly 1 | 326·0 | 182·2 | 94·4 | 400·8 | 290·5 | 125·7 |
| CD8 | 238·0 | 118·6 | 55·9 | 245·9 | 160·0 | 58·2 |
| Thy-1 + Ly 1 | 250·7 | 151·0 | 73·1 | 421·8 | 276·2 | 102·1 |
| CD4 + CD8 | 64·3 | 25·8 | 9·1 | 54·2 | 13·9 | 4·0 |

* Specificities of the antibodies used are shown (see the Materials and Methods). The top five antibodies are IgG2b, the next three are IgG2a, and the final combinations each contain both subclasses. All antibodies were added at the start of culture and were used as supernatants at approximately 10% (vol/vol); this was shown to be a saturating dose, giving a plateau of inhibition, when titrated in several experiments (not shown). Antibodies F4/80 and KJ16 were tested in other experiments with similar results to 2.4G2.

† The indicated doses of C57/BL10 DC were cultured with $2·5 \times 10^5$ periodate-modified syngeneic T cells and the antibodies shown, and pulsed with thymidine from about 28–42 hr: background proliferation was $7·1 \times 10^3$ c.p.m.

‡ The indicated doses of C57/BL10 DC (same preparation as used in oxidative mitogenesis) were cultured with $3·5 \times 10^5$ BALB/c T cells and the antibodies shown, and pulsed with thymidine from about 72–78 hr: background proliferation was $1·7 \times 10^3$ c.p.m.

### Early clustering of dendritic cells and macrophages with periodate-modified T cells is inhibited by antibodies to CD18 but not to CD4 and CD8

To ask which molecules may be involved in DC and Mϕ clustering, we tested the effect of a panel of antibodies in the rapid cluster assays. The only antibody tested that inhibited clustering, for both DC and Mϕ, was CD18 (Table 4). The

**Table 2.** Time-course of inhibition of oxidative mitogenesis and the allogeneic MLR by CD18 and CD4 antibodies

| | Response (c.p.m. × $10^{-3}$) in presence of antibody to: | | | | | |
|---|---|---|---|---|---|---|
| | Experiment 1 | | | Experiment 2 | | |
| Time (hr)* | FcR | CD18 | CD4 | FcR | CD18 | CD4 |
| Oxidative mitogenesis† | | | | | | |
| 0 | 175·6 | 1·7 | 99·1 | 293·7 | 11·2 | 147·0 |
| 5 | — | — | — | 260·9 | 70·3 | 165·6 |
| 10 | 172·7 | 67·8 | 142·6 | — | — | — |
| 20 | 172·6 | 124·7 | 154·7 | 267·6 | 217·5 | 248·4 |
| 25 | — | — | — | 249·8 | 270·4 | 295·6 |
| 30 | 149·1 | 163·7 | 157·8 | — | — | — |
| 35 | 156·7 | 156·0 | 149·7 | — | — | — |
| Allogeneic MLR‡ | | | | | | |
| 0 | 118·4 | 41·1 | 41·4 | 88·7 | 5·8 | 54·0 |
| 24 | 124·2 | 121·4 | 91·8 | 122·2 | 23·0 | 76·7 |
| 48 | 141·9 | 132·6 | 134·5 | 84·0 | 53·3 | 89·9 |
| 72 | 155·3 | 169·3 | 156·3 | 102·7 | 61·4 | 105·9 |

* At the indicated times (± 1 hr) the antibodies as shown were added to the cultures (see legend Table 1). Other control antibodies were also tested in Exp. 1 and did not inhibit.

† C57/BL10 (Exp. 1) or BALB/c (Exp. 2) DC were cultured with $3 \times 10^5$ syngeneic periodate-treated T cells at 1:30 (Exp. 1) or 1:50 (Exp. 2). 0–25-hr cultures were pulsed with tritiated thymidine from 25–39 hr, and 30–35 hr cultures were pulsed 24 hr later: background proliferation was 1·1 and $10{\cdot}1 \times 10^3$ c.p.m. for Exp. 1 and 2, respectively.

‡ DC (strains and ratios as for oxidative mitogenesis) were mixed with untreated allogeneic BALB/c or C57/BL10 T cells. Cultures were pulsed with tritiated thymidine from 92 to 107 hr: background proliferation was 14·1 and $5{\cdot}5 \times 10^3$ c.p.m. for Exp. 1 and 2, respectively.

**Table 3.** Syngeneic and allogeneic DC and Mϕ cluster with periodate-treated T cells at 37°, but not at 4°

| | | % clustering with: | | | |
|---|---|---|---|---|---|
| Temp. | Ratio* T:DC/Mϕ | DC+ syn. T† | DC+ allo. T‡ | Mϕ+ syn. T† | Mϕ+ allo. T‡ |
| 37°§ | 2:1 | 54 | 49 | 54 | 61 |
| | 4:1 | 45 | 46 | 47 | 58 |
| | 8:1 | 45 | 38 | ND | 38 |
| | 16:1 | 30 | 30 | 11 | 13 |
| 4°¶ | 2/4:1 | 15 | 2 | 13 | 15 |

* DC were added in graded numbers to a constant number of periodate-treated T cells; one experiment is shown. In other experiments similar results were obtained using up to 1:1 Mϕ:T.

† BALB/c DC or Mϕ and BALB/c T cells.

‡ C57/BL10 DC or Mϕ and BALB/c T cells.

§ Clusters at 37° contained 5–>30 lymphocytes.

¶ Clusters at 4° contained only 1–5 lymphocytes.

ND, not done.

**Table 4.** CD18, but not CD4 and CD8 antibody, inhibits clustering of DC and Mϕ with periodate-treated T cells

| | % clustering† | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DC+syn T | | | | Mϕ+syn T | | | |
| Antibody* specificity | Exp. 1 | Exp. 2 | Exp. 3 | Exp.4 | Exp. 4 | Exp. 5 | Exp. 6 | Size‡ |
| Thy-1+Ly 1 | 60 | ND | ND | 68 | 84 | 68 | 62 | 5–>30 |
| Thy-1+LCA | 61 | 61 | 73 | ND | ND | ND | ND | 5–>30 |
| CD4+CD8 | 56 | 63 | 69 | 51 | 78 | 47 | 65 | 5–>30 |
| CD18 | 28 | 47 | 37 | 32 | 36 | 32 | 20 | 1–5 |

* As for Table 1. Other antibodies in that table were tested but did not inhibit clustering.

† DC and Mϕ were mixed at ratios of from 0·3:1 to 1:1 with T cells. C57/BL10 cells were used in Exps 1 and 6, BALB/c in others. DC in Exp. 1 were further purified before use by depleting Fc-rosetting cells as described elsewhere (Steinman, Van Voorhis & Spalding, 1985).

‡ Number of lymphocytes in clusters.

ND, not done.

actual percentage of clustered cells was reduced to about half of that with control antibodies, but the size of the remaining clusters was also drastically reduced (Table 4). Although CD4 and CD8 antibodies inhibited proliferation (Table 1) they had no effect on clustering, alone (not shown) or in combination (Table 4).

CD18 inhibited clustering whether it was added immediately to the cells or preincubated with them for an hour at 4° before the assay. We asked whether its effect was on the DC or on the T cell. (By analysis on an Ortho Cytofluorograph, DC express very low levels of CD18 antigen; not shown.) DC and T cells were separately preincubated with CD18 (or control anti-LCA) for 45–60 min. They were then washed to remove unbound antibody before setting up the assays with the different cell combinations. In three experiments clustering was inhibited when both DC and T cells were preincubated with CD18, but it was not if only the DC were treated; thus we favour an action of CD18 on the T cell rather than the DC. However, this is tentative since clustering was inhibited in only two of the three experiments when the T cells but not the DC were preincubated.

In summary, CD18 antibodies can inhibit cluster formation with both DC and Mϕ, but only the former induce mitogenesis of periodate-modified T cells.

## DISCUSSION

Elsewhere we showed that clustering with DC precedes and is essential for T-cell proliferation in the periodate model (Austyn *et al.*, 1988). We have now used this system to examine some of the molecules responsible both for accessory cell–T-lymphocyte clustering and T-cell activation. Several new findings are reported here.

Firstly, it has been assumed that MHC class II (Ia) expression and IL-1 activity are critical for T-cell induction. But clustering with a cell that can express Ia molecules and synthesize IL-1, the Mϕ, is not sufficient for mitogenesis (Figs 1 and 2). In addition, Ia expression is not sufficient for clustering of periodate-treated T cells, since B cells and B blasts do not

cluster or stimulate proliferation (Fig. 3). In subsequent experiments (not shown) we have found that Mϕ cultured in interferon-gamma to induce high levels of Ia behave similarly to the untreated Mϕ used here. Furthermore, Koide, Inaba & Steinman (1987) have shown that they do not induce proliferation of allogeneic T cells, even in the presence of exogenous IL-1. Since DC possess Ia but in any case do not synthesize IL-1 (Koide & Steinman, 1987), there must be other components responsible for their stimulatory capacity.

Secondly, proliferation in both oxidative mitogenesis and the MLR was inhibited by CD18, CD4 and CD8 antibodies (Table 1). In this respect the two responses were similar, but the MLR was less sensitive to CD18. The additive or slightly synergistic effects of CD4 and CD8 antibodies (Table 1) suggests that both MHC class I- and class II-restricted T cells respond in oxidative mitogenesis. In preliminary experiments we found that polyclonal antisera to both classes of molecules can inhibit this response (J. M. Austyn, D. E. Weinstein and R. M. Steinman, unpublished observations); Phillips *et al.* (1980) also found inhibition by anti-Ia antisera. Both CD18 and CD4 antibodies were most inhibitory when added initially to the cultures, but they had no effect once DNA synthesis had commenced (Table 2), suggesting they are involved here principally in primary T-cell activation. We also found that the anti-Thy-1 antibody was, for reasons unknown, sometimes inhibitory; certain anti-Thy-1 antibodies stimulated proliferation, particularly of T-cell clones and hybridomas (Guner *et al.*, 1986), but to our knowledge they were not inhibitory.

Thirdly, we found that both syngeneic and allogeneic DC and Mϕ could cluster with periodate-treated T cells in a temperature-dependent manner (Table 3). Elsewhere we have shown that this response is not MHC restricted, as expected for a true polyclonal system, and that proliferation can be stimulated by syngeneic and allogeneic DC. This was also found in another species (Greineder & Rosenthal, 1975). Clustering of both DC and Mϕ was dramatically inhibited by CD18 (Table 4). Even so, it was not complete, implying that other molecules may also mediate such cellular associations. Thus, there are clear similarities between early clustering with DC and Mϕ, but the Mϕ–T-cell clusters are unstable and eventually dissociate spontaneously after several hours (Fig. 1). The CD4 and CD8 antibodies did not affect clustering (Table 4), despite their inhibitory effects on mitogenesis (Table 1). These molecules seem to be involved in the stages of T-cell activation that follow clustering, whether or not they mediate an 'off' signal to the T cell, as suggested by some, and/or interact with MHC molecules as suggested by others.

Finally, we found that CD18 probably inhibits at the level of the T cell, but not the accessory cell. Similarly, we showed the same in another polyclonal response, that of human T-cell activation by CD3 antibodies (Austyn, Smith & Morris, 1987), where we used mouse Mϕ and B blasts as FcR-bearing accessory cells and found that they were inhibited by human CD18 antibodies but not by the same mouse CD18 antibody used in the present study. We think it is likely that the multitude of inhibitory effects reported for CD18 antibodies in T-dependent responses may be exerted generally at this level. If so, this raises the question of which molecules on the accessory cell are involved in clustering. We presume that these have yet to be defined.

Rapid cluster assays have been used by others to define antigen-dependent and -indepedent phases of clustering of sensitized T cells: the former can proceed at 4° with any accessory cell but the latter appears to occur uniquely with DC at 37° (Green & Jotte, 1985; Inaba & Steinman, 1986). However, clustering can not be examined in antigen-specific responses before a day or so of culture, whereas cluster formation in oxidative mitogenesis is rapid and by 3 hr essentially all the T cells that can cluster have done so. During preparation of this paper, Inaba & Steinman (1987) reported that the CD18 antibody inhibited proliferation in the MLR but, in marked contrast to oxidative mitogenesis, it did not inhibit the formation of clusters although it destabilized them; we have also noted this phenomenon (unpublished observations). Thus, oxidative mitogenesis and the MLR differ in their dependence on the CD18 molecule since proliferation and clustering are inhibited to differing degrees, and the inhibition becomes less with increasing doses of DC in the MLR but not in oxidative mitogenesis (Table 1). Most probably CD18 and other molecules play differential roles in cellular adhesion during these responses. CD4 and CD8 antibodies did not influence clustering in oxidative mitogenesis (Table 4), nor during antigen-dependent binding in the MLR (Inaba & Steinman, 1987), but Green & Jotte (1985) observed that CD4 did block binding of memory cells in the rat MLR.

Oxidative mitogenesis can proceed whether the T cell and/or the accessory cell is treated. For the latter, some have suggested that periodation introduces active aldehyde groups that lead to association of normal membrane components with adjacent MHC molecules (Keren & Berke, 1986). These might create neoantigenic determinants that are recognized by T cells as a form of alloantigen. However, we routinely modified the T cells rather than the DC. In this case rapid 'cross-linking' of surface components, such as CD3–T-cell receptor complex, may prime the cell to respond sooner than would normally occur in response to antigen. One consequence of this may be activation of CD18-dependent adherence. Others have shown that anti-CD18 antibodies can inhibit the reaggregation of dissociated periodate-modified cells (Hamann, Jablonski-Estrich & Thiele, 1986). Furthermore, treatment of the cells with reducing agents does not prevent their association, showing that the continuous presence of mitogen is unnecessary.

In summary, we have shown that DC and Mϕ, but not B cells or B blasts, can cluster with periodate-treated T cells. Clustering in both instances is a temperature-dependent process in which the CD18 molecule plays a prominent role, probably at the level of the T cell. Since only clustering with DC leads eventually to proliferation, we conclude that CD18-dependent clustering is not sufficient for T-cell mitogenesis. By implication, other molecules are required for the unique capacity of DC to stimulate primary T-cell responses.

## ACKNOWLEDGMENTS

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