Clin. exp. Immunol. (1986) **66,** 118–125

Studies on the accessory requirement for T lymphocyte activation by Concanavalin A

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(Accepted for publication 21 May 1986)

SUMMARY

In this study we have examined the interactions between accessory cells (AC) and T cells in response to Con A. Highly purified peripheral blood T cells and AC exposed to a variety of treatments were used. We found that untreated AC provided optimal help for T cell proliferation and this was not mediated by soluble factors since whole cells could not be replaced with supernatants from activated AC. Furthermore, cycloheximide-treated AC were able to supply the accessory signal although unable to elaborate soluble activation factors. To find out more about the accesory signal, we examined the ability of monocytes mildly fixed with glutaraldehyde to supply help. These cells were completely unable to perform as AC, although they were viable and had unaltered surface antigen expression. They could not secrete activation factors, but this alone could not explain their inability to supply help because this function was not restored with the addition of soluble activation factors. This indicated that AC-T cell contact was of prime importance to accessory function. To investigate the possibility that AC work by cross-linking structures on the lymphocyte surface, we attempted to substitute for the soluble Con A plus AC with Con A bound to the surface of erythrocytes. Comparable stimulation was observed, suggesting that the cross-linking of Con A-bound structures on the lymphocyte surface generates the accessory signal.

Keywords Concanavalin A lymphocyte activation accessory cells

INTRODUCTION

The study of mitogen-induced T cell proliferation has been of central importance to the understanding of lymphocyte responsiveness, particularly the early events in activation. Con A is capable of binding to the lymphocyte membrane (Larsson & Coutinho, 1979) thereby inducing sensitivity to interleukin 2 (IL-2) (Larsson, Iscove & Coutinho, 1980), although this alone is not sufficient for activation. Current models propose that both mitogen and a second signal, derived from an accessory cell (AC), are required for T lymphocyte expression of IL-2 receptors and for the synthesis and secretion of IL-2 (Klaus & Hawrylowicz, 1984). Proliferation of lymphocytes follows these early activation events.

The nature of the AC-derived signal is not yet fully understood. However, it is clear that the mechanism is not analogous to antigen presentation in association with class II MHC molecules, because allogeneic AC function as efficiently as syngeneic AC (Rock, 1982). Furthermore, Ia^- cells

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have recently been shown to be capable of supplying the accessory signal (Roska, Johnson & Lipsky, 1984). It has been suggested that soluble factors derived from activated AC transmit the accessory signal and may be used in place of whole cells. This is a controversial idea with papers with both supporting (e.g. Maizel *et al.*, 1981) and refuting it (e.g. Hunig, 1983). A further possibility is that the transmission of the signal requires cell-cell interaction (Hunig, 1983).

In these studies we have tried to define more clearly the accessory signal in the response to Con A. The ability of AC, treated in a variety of ways, to supply signals for the induction of proliferation of highly purified T cells has been assessed, as has the capacity of soluble factors to replace or enhance AC. We have also examined a method of substituting for AC using Con A attached to the surface of erythrocytes.

MATERIALS AND METHODS

Cell preparation. Peripheral blood mononuclear cells were obtained by Ficoll-paque (Pharmacia) density separation. T cells were prepared by a further three-step purification procedure. The first was incubation in plastic tissue culture dishes. Secondly, nonadherent cells were then enriched for T cells by passage through two nylon wool columns. Finally, cells eluted from the second column were incubated with anti-HLA-DR monoclonal antibody (Becton Dickinson) followed by baby rabbit complement. This resulted in a purified T cell population, >96% staining with a pan-T monoclonal antibody (anti-T3, Ortho Diagnostics).

Adherent cells recovered from the culture flask were used as the accessory cell population and were > 70% monocytes as judged by fluorescence using the monocyte-specific monoclonal antibody Mo2 (Coulter).

Treatment of accessory cells. AC were exposed to 2,500 Rads from a gamma radiation source, to prevent their proliferation. They were then used as untreated AC, or exposed to one of two further treatments. Mild fixing was achieved by treatment with 0·1% glutaraldehyde for 10 s followed by addition of 5 × volume of PBS. Cells were washed 3 × before use. Incubation in cycloheximide solutions (10 or 1000 μM) (Sigma) was carried out for 1 h at 37°C. Again cells were washed three times before use.

Preparation of activated accessory cell supernatants. This was based on the method of Palacios (1982). Briefly, 5×10^6 T cells were cultured with 5×10^6 AC in 2 ml in the presence of 10 μg/ml concanavalin A. After 12 h at 37°C the cells were washed three times and cultured for a further 24 h without Con A. The supernatants were collected and their activity checked using a mouse thymocyte proliferation assay (Palacios, 1982). In this assay 5×10^5 mouse thymocytes were cultured in 200 μl, including 50 μl of the supernatant under test. The cells were cultured for 2 days, with tritiated thymidine (^{3}HTdR) (Amersham) added during the final 12 h. Proliferation was measured by liquid scintillation counting. Supernatants were stored at −20°C until use.

Culture condition. T cells (2×10^5) were cultured with or without 4×10^4 AC, in the presence or absence or 200 μl in concanavalin A. These were cultured for 3 days and 0·3 μCi ^{3}HTdR (specific activity 2·0 Ci/m mole) in 10 μl was added for the final 18 h of culture. The uptake of ^{3}HTdR of culture. The uptake of ^{3}HTdR was measured by liquid scintillation counting.

The same conditions applied to the culture of T cells with erythrocytes, using erythrocyte to T cell ratios of 10:1 or 100:1.

Preparation of Con A-coated erythrocytes. Packed human red blood cells were washed five times before use and buffy coat cells drawn off and discarded. Samples of packed human red blood cells, 240 μl, were resuspended in 120 μl of protein solution. Slowly, while, vortexing, 600 μl chromic chloride solution (0·1 mg/ml) was added and tubes were left overnight at 4°C. Cells were washed and resuspended at 2·5% v/v. A range of Con A solutions from 1 μg/ml to 20 mg/ml was used and binding was verified using anti-Con A antisera to agglutinate coated red cells. Bovine serum albumin was attached to red cells for use as a control.

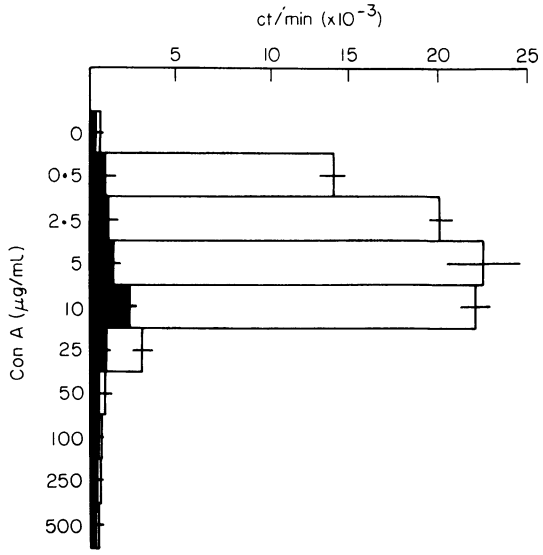

Figure 1. AC requirement for response to Con A. T cells cultured with or without AC. Results show mean ± s.d. for uptake of ^{3}HTdR. With AC (□); without AC (■).

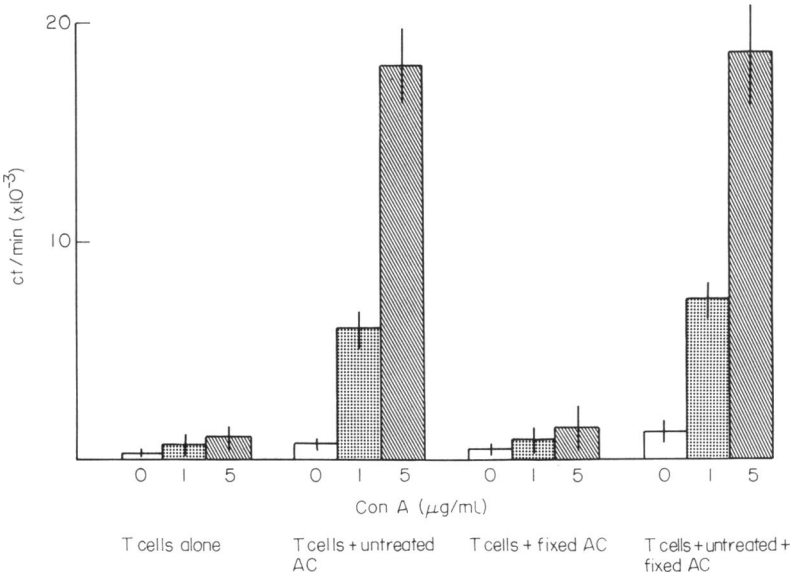

Figure 2. Fixed cells cannot supply accessory function. Uptake of ^{3}HTdR by T cells cultured alone or with untreated or fixed AC. Results shown are mean ± s.d.

RESULTS

The requirement for accessory cells in the T cell response to Con A. We studied the proliferation of T cells cultured with a wide range of Con A concentrations in the presence and absence of irradiated AC. The optimal ratio of T cells to AC (4:1) was used. This ratio gave the highest levels of proliferation in preliminary experiments using a variety of T cell:AC proportions (results not shown). As shown in Fig. 1, substantial proliferation is seen at lower mitogen concentrations when irradiated monocytes are included in the culture. At concentrations of 25 μg/ml and above, Con A inhibits proliferation. T cells cultured in the absence of AC were unable to proliferate over the entire range of Con A concentrations. This demonstrates the need for an accessory signal in Con A-induced T cell proliferation. In subsequent experiments, Con A concentrations of 1 or 5 μg/ml were used.

It was noted that in those cultures containing AC, marked aggregation of the cells occurred. This clumping was most marked in cultures containing Con A but was also present in cultures without Con A.

The inability of fixed cells to supply accessory signals. Glutaraldehyde-fixed cells were used to examine the role of AC in the response to Con A (Fig. 2). These mildly fixed cells were unable to supply the accessory signal, the level of uptake of ^{3}HTdR being no different to that measured when T cells were cultured alone. In the presence of untreated, AC, substantial proliferation was seen, as before. When untreated and fixed AC were added together, a large proliferative response was measured, demonstrating that T cells were not inhibited from responding by the fixed AC but rather that fixed AC were unable to supply the accessory signal.

In cultures containing fixed AC, no aggregation of cells was observed during culture, in contrast to cultures with untreated AC. Where fixed and untreated AC were cultured together with T cells, clumping occured.

The conditions of fixing (0·1% glutaraldehyde for 10 s) were the minimum requirements for interference with accessory function. After treatment at a lower concentration (0·05% glutaraldehye), the cells had maintained full accessory capacity. Under both these conditions of fixing, the viability of the cells was equal to that untreated cells, as judged by ethidium bromide–acridine orange staining. Futhermore, cell surface antigen expression (as detected by the monocyte-specific monoclonal antibody, Mo2) on the fixed cells was identical, both in numbers of cells staining and in pattern of staining, to untreated accessory cells.

Production of activation factors by accessory cells. Soluble factors, produced by activated AC may be important mediators of accessory function in the T cell response to Con A. One method of measuring these factors is to determine their ability to support the proliferation of mouse thymocytes (Palacios, 1982). The results of such a thymocyte proliferation assay are given in Table 1. In the absence of supernatant, thymocytes failed to proliferate. The addition of supernatant produced by untreated AC supported proliferation of the thymocytes, but supernatants from fixed or cycloheximide-treated AC had no effect on the thymocytes.

Table 1. Thymocyte proliferation assay

Supernatant source	ct/min
No supernatant	80 ± 27
Irradiated AC	1021 ± 164
Fixed AC	90 ± 19
Cycloheximide-treated AC	187 ± 69

Mouse thymocytes cultured in 200 μl containing 25% supernatant. Results shown are mean ± s.d. of triplicate cultures.

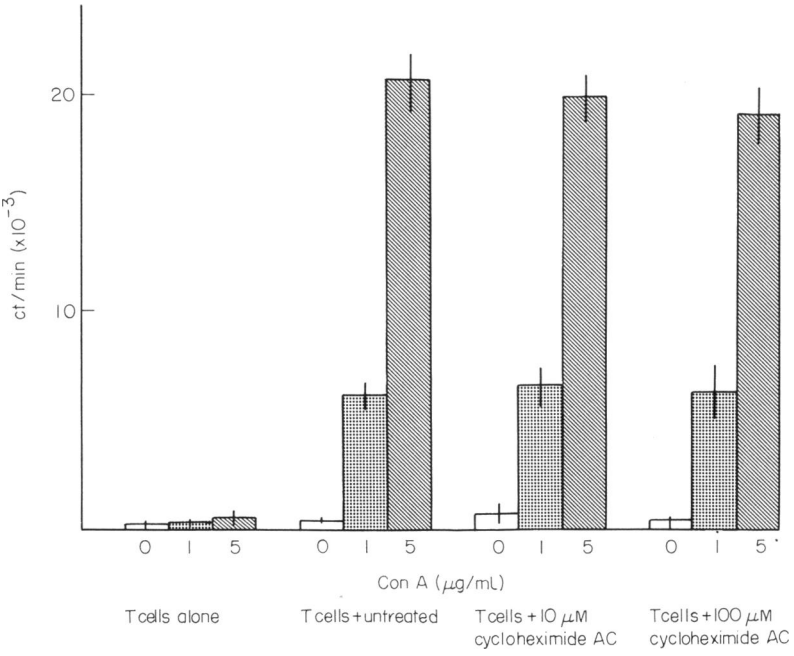

Figure. 3. Effect of cyclocheximide on accessory function. Uptake of ^{3}HTdR. Results show mean ± s.d.

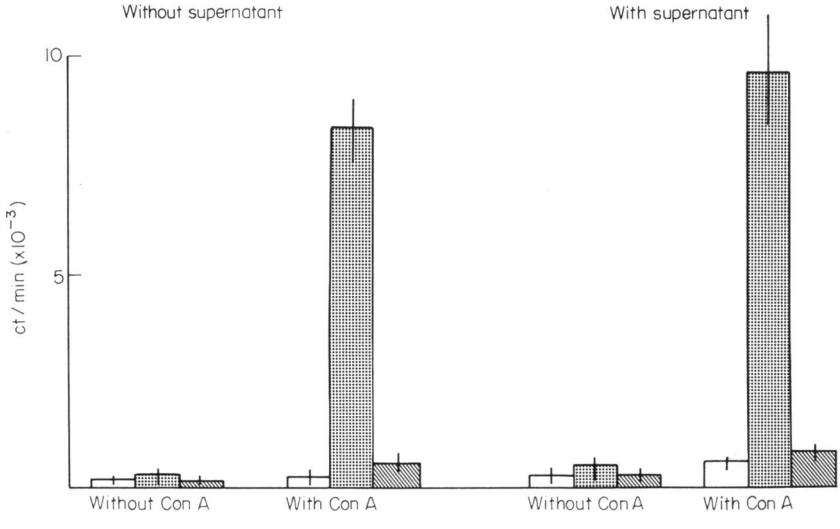

Figure 4. The effect of activated supernatants on AC function. Results show mean ± s.d. for uptake of ^{3}HTdR. T cells alone (□); T cells + untreated AC (▨); T cells + fixed AC (■).

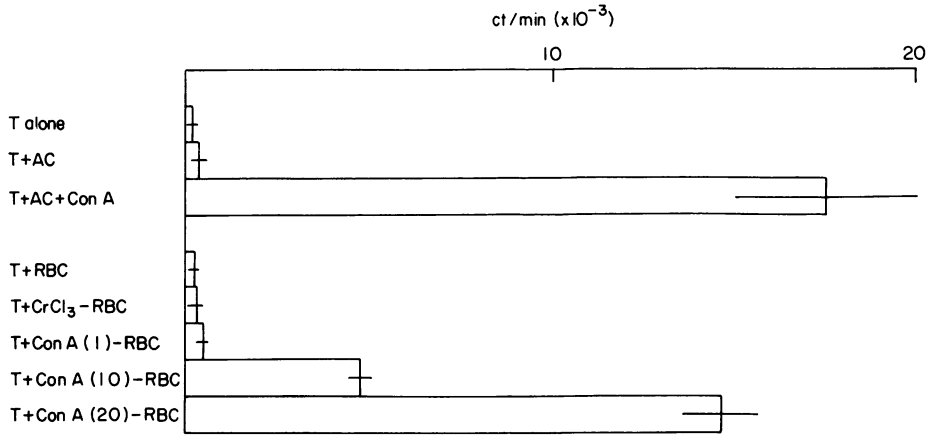

Figure 5. Replacement of AC with Con A-coated erythrocytes. RBC cultured with T cells at a ratio of 100:1. (1), (10) and (20) refer to concentrations of Con A solutions in mg/ml used in attaching Con A to erythrocytes. Results shown are mean ± s.d. values for uptake of ^{3}HTdR.

Accessory function of fixed cells is not restored by soluble factors. The inability of fixed AC to secrete factors capable of inducing mouse thymocyte proliferation (Table 1) may have explained their ineffectiveness in supplying the accessory signal. To test this idea, experiments were performed in which T cells and fixed AC were cultured with Con A in the presence of activated accessory cell supernatants. These supernatants, which showed activity in the mouse thymocyte proliferation assay (Table 1), constituted 50% of the final culture volume. The results (Fig. 3) show that the activated supernatants were unable to restore accessory function to fixed cells. Furthermore, these supernatants did not enhance the response seen in the presence of untreated AC. The addition of activated accessory cell supernatants had no effect on the ability of AC to induce aggregation of T cells; untreated cells maintained this ability and fixed cells did not acquire it.

Efficacy of cychoheximide-treated accessory cells. In order to study further the role of soluble factors in the response, AC were treated with cycloheximide and tested for the ability to provide accessory function. As shown in Fig. 4, AC treated with 10 μM or with 1 mM cycloheximide provided accessory function as effectively as irradiated AC. Cycloheximide-treated cells also maitained the capacity to induce aggregation in culture.

Cross-linked Con A activate cells in the absence of Con A. It has previously been suggested that specific receptor-ligand interactions are important in AC function (Bekoff, Kakiuchi & Grey, 1985). In this study, experiments were designed to test the hypothesis that AC work by cross-linking Con A bound to glycoproteins on the lymhocyte surface. Erythrocytes were used as an inert carrier and Con A was attached to their surface using chromic chloride. A wide range of Con A concentrations was used and the Con A-coated erythrocytes were cultured with T cells at ratios of 10:1 and 100:1. No proliferation was measured in any of the cultures using the 10:1 erythrocyte:T cell ratio. Figure 5 shows results from a representative experiment. In response to Con A-coated erythrocytes, reproducible concentration-dependent stimulation of T cells was seen. Using erythrocytes with maximal Con A attached, this stimulation was as high as that obtained using T cells, AC and soluble Con A at optimal concentrations. No proliferation was observed in cultures containing T cells plus untreated erythrocytes, T cells plus chromic chloride-treated erythrocytes or T cells plus BSA-coated erythrocytes.

To test the possibility that RBC preparations were contaminated with viable accessory cells, purified T cells were cultured with chromic chloride-treated RBc and soluble Con A. No proliferation above background was measured in these cultures (results not shown).

DISCUSSION

Habu and Raff (1977) were the first to suggest the accessory requirement for mitogenic T cells responses, demonstrating the need for an Ia^+ accessory cell. Since then, there have been conflicting views on whether accessory cells act via soluble factors or function through cell-cell interaction. The results presented in this study support the second of these possibilities.

It was shown here that while whole cells supply accessory signals, soluble factors from activated AC cannot induce the proliferation of highly purified T cells in the presence of Con A. This finding is in agreement with some previous studies on mitogen responsiveness (Lipsky, Ellner & Rosenthal, 1975; Hunig, 1983) but is in contrast to the studies of Palacios (1982) and of Maizel *et al.* (1981) who found that soluble factors fully substituted for cells. These differences in accessory requirement may simply reflect the purity of the T cell population used, since a small number of contaminating monocytes may supply accessory function. In this work, T cells were purified by three sequential procedures: plastic adherence, nylon wool adherence and antibody-mediated complement lysis. If any one of these three steps was omitted, proliferative responses to Con A were demonstrable in the absence of added AC (results not shown). We attribute this to low levels of contaminating monocytes supplying accessory function.

In contrast to others (Roosnek, Brouwer & Aarden, *et al.*, 1985), these experiments show that the addition of soluble factors to cultures containing AC did not enhance proliferative responses. This may be due to differences in culture conditions, for example the % monocytes added or the concentration of Con A used. Our culture system used optimal conditions, perhaps precluding enhancement by soluble factors. Soluble factors, however, may enhance suboptimal responses, as has been demonstrated for IL-2 and thiols by Roosnek *et al.* (1985).

Examination of the cultures throughout incubation showed that aggregation of the cells in culture was intimately associated with proliferation. Cycloheximide-treated AC were capable of inducing both aggregation and proliferation, while fixed cells could achieve neither. This suggested that AC supplied the accessory signal via an interaction with lymphocyte surface structures. This signal may have been transmitted through structures bound by Con A or via independent structures. There is little evidence to allow discrimination between these possibilities. T cell activation can be inhibited by antibodies directed at structures distinct from the antigen receptor, e.g. Lyt-2 (Hollander, Pillemer & Weissman, 1980), L3T4 (Swain *et al.*, 1984) and LFA7 (Pierres, Goridis & Goldstein, 1982). However, it is not known whether Con A binds to these structures or if these structures bind ligands on the AC surface. We tested the first proposal, employing Con A attached to the surface of erythrocytes. The success of this approach at high surface concentrations of Con A and at large RBC:T cell ratios supports the idea that the accessory signal is delivered via a Con A-bound structure. Furthermore, in cultures of T cells with Con A-coated RBC which resulted in high levels of proliferation, clustering patterns were noted during incubation. This was dependent on T cells because when RBC were cultured alone a smooth lawn was seen.

It appears, therefore, that the accessory cell requirement in the response to Con A is not absolute, but a reflection of culture conditions. At low concentrations of soluble Con A, whole AC are required. These accessory cells very efficiently cross-link structures on the lymphocyte surface bound by Con A. Under suboptimal conditions, this may be enhanced by soluble activation factors secreted from AC. To completely replace AC, very high concentrations of immobilized Con A are required. We suggest that the activating signal is generated in the same way, i.e. by the cross-linking of structures bound by Con A.

In conclusion, we have shown that in the T cell response to Con A, the required accessory signal is supplied by whole cells which cannot be replaced by soluble factors derived from the same population. Aggregation of the cells is intimately involved in activation. An alternative method for supplying the accessory signal, using Con A attached to RBC, also works via an aggregation step. Thus, the ability to supply the accessory signal is not a feature unique to whole cells, but be induced by different methods of cross-linking structures on the lymphocyte surface bound by Con A. Con A stimulation of T lymphocytes, therefore, bears little resemblance to current understanding of antigenic activation, where viable, syngeneic, Ia^+ accessory cells are a prerequisite.

R.B.G. was supported by a Research Fellowship from the National Research Council of Ireland.

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