THE SPECIFIC DIRECT INTERACTION OF HELPER T CELLS AND ANTIGEN-PRESENTING B CELLS

II. Reorientation of the Microtubule Organizing Center and Reorganization of the Membrane-associated Cytoskeleton inside the Bound Helper T Cells

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Th cells form a major branch of effector T cells that regulate the immune response. A principal function of Th is to stimulate antigen-presenting B cells to proliferate and differentiate into antibody-producing cells. Th cells possess a clonally distributed T cell receptor that recognizes a specific antigen (Ag)[1] in the context of a particular allele of the MHC class II molecules (Ia in the mouse) displayed on the surface of an APC. Although it has long been suspected that B cell activation occurs as a consequence of a specific interaction between Th and B cells, direct evidence for such an interaction has been lacking. In the first paper of this series, we have recently shown (1) that 1:1 cell couples could be formed between an Ag- and Ia-specific cloned Th cell and an Ag-pulsed B hybridoma cell; shortly afterwards, as seen by immunofluorescence experiments, the microtubule organizing center (MTOC) inside the Th cell, but not the B hybridoma cell, was oriented towards the region of cell–cell contact. The induction of this MTOC orientation in the Th cell was shown to be Ag- and Ia-specific, and thus provided the first demonstration of a direct specific Th–B cell interaction.

The MTOC in T cells, as in almost all other types of eukaryotic cells in interphase, is a fairly compact structure situated to one side of the cell nucleus (see reference 2). Colocalized with the MTOC is the compact Golgi apparatus (GA), the key structural element in the processing and transport of secretory components by the cell. A coordinate reorientation of the GA/MTOC inside cells often follows the receipt of a polarized signal by the cell (2–5). It was postulated (1) that the function served by the observed orientation of the MTOC inside Th cells bound to their specific Ag-presenting B cells was to direct GA-derived secretory vesicles, containing putative B cell growth factors and lymphokines, for fusion and secretion at the region of contact with the B cell.

In this paper, these investigations have been pursued in several important directions: (a) The specific MTOC (and presumably GA) orientation was shown to occur in two additional Th–B systems besides the one originally used; (b) Ag processing by the APC was shown to be required for the specific MTOC/GA orientation to occur; (c) specific allogeneic Th–B interactions (6) were found also to induce the MTOC/GA orientation; (d) the cytoskeletal protein talin was shown to become concentrated inside the Th cell at the region of specific binding to the APC, as had previously been found (5) with CTL interacting with their specific target cells; and (e) the MTOC orientation accompanying specific Th–B cell binding depended on extracellular Ca^{2+}, but the talin redistribution did not.

Materials and Methods

Cells. The cloned Th cell lines D8 and D10.G4.1 (D10) specific for the antigens Ova and Con, respectively, in conjunction with Ia^k, have been described elsewhere (6). These were the gifts of Dr. Charles A. Janeway, Jr., Department of Pathology, Yale University School of Medicine, New Haven, CT. 2H10.H1 is a subclone of 2H10, a T–T hybridoma with a helper response specific for pigeon cytochrome *c* (Cyt) and Ia^k (7). The B cell hybridomas, LK and LB, express Ia^d and either Ia^k (LK) or Ia^b (LB) on their cell surfaces (8). The B cell lymphomas BCL1 (Ia^d) (9) and CH12 (Ia^k) (10) were carried as in vivo tumors in mice. The T cells were maintained as described elsewhere (6). The B cell hybridomas were maintained in high-glucose DME supplemented with 10% FCS.

Antibodies. The mouse mAb MKD6, specific for Ia^d, was used as before (1). Fluorescein-tagged $F(ab')_2$ fragments of goat anti–mouse IgG were obtained from Cooper Biomedical, Inc., Malvern, PA. The affinity-purified rabbit antibodies specific for chick brain tubulin, and the affinity-purified rabbit antibodies specific for each of the cytoskeletal proteins α-actinin, talin, and vinculin were as used in our previous studies (5).

Cell Conjugation and Immunofluorescence Labeling. The D8 and D10 cells were used about 2 wk after they were last stimulated with Ag. The B cell lymphomas or hybridomas were incubated overnight with any one of the Ags Con (500 μg/ml), Ova (500 μg/ml), or Cyt (200 μg/ml). After washing away the unbound Ag, the B cells were mixed with the appropriate T cells at a 1:1 ratio. The cell mixtures were centrifuged for 5 min at 500 rpm, and after 10 additional minutes of incubation at 37°C, the cell mixtures were resuspended. Aliquots containing ~10^5 cells were plated on poly-L-lysine–treated coverslips, and fixed with 3% formaldehyde. In the experiments involving the B cell hybridomas LK or LB, the cell mixtures were treated with the MKD6 antibodies, to mark the B cells, before fixation. In the experiments in which the B cell lymphomas BCL1 and CH12 were used, the fixed cells were labeled with fluorescein-tagged $F(ab')_2$ fragments of goat anti–mouse IgG to mark the B cells, which are Ig^+. The membranes of the fixed cells were permeabilized by brief treatment with 0.15% Triton- X-100, and the cells were further labeled with rabbit antibodies specific to any one of the cytoskeletal proteins tubulin, α-actinin, talin, or vinculin. The cells were doubly labeled with rhodamine-modified $F(ab')_2$ fragments of goat anti–rabbit IgG and with fluorescein-tagged $F(ab')_2$ fragments of goat anti–mouse IgG. The immunofluorescently labeled cells were viewed with a photoscope III (Carl Zeiss, Inc., Thornwood, NY) as in previous studies (2, 5).

Results

Generality of the MTOC Reorientation in Specifically Bound Th Cells. When the Ag-presenting CH12 (Ia^k) B-lymphoma cells were pulsed overnight with the specific Ag Ova and then mixed with an equal number of D8 Th cells, numerous cell couples were formed between them (Fig. 1 C). Immunofluorescent microscopic observations of these specific cell couples indicated that the MTOC inside essentially all (>95%) of the bound D8 cells was oriented toward the contact

region with the CH12 cell, but the MTOC in these CH12 cells remained randomly oriented (Fig. 1 A).

If the CH12 cells were pulsed overnight with the nonspecific Ag Cyt and then mixed with the D8 cells, or if the BCL_1 (Ia^d) B-lymphoma cells were pulsed with the specific Ag Ova and then mixed with the D8 cells, stable cell couples were formed in both cases (only the former shown in Fig. 1, D–F). On morphological grounds alone, as observed in Nomarski optics, the specific cell couples (Fig. 1 C) could not be clearly distinguished from the nonspecific ones (Fig. 1 F). However, the MTOC inside the D8 cells in such nonspecific couples was randomly oriented; in ~55% of the couples, the MTOC faced away from the contact with the Cyt-pulsed CH12 cell (as in Fig. 1 D) or with the Ova-pulsed BCL_1 cell (data not shown, but as in Fig. 1 D).

Similar results were obtained using the Th hybridoma 2H10 specific for Cyt plus Ia^k. When CH12 cells pulsed overnight with Cyt were mixed with 2H10 cells, many 1:1 cell couples were formed (Fig. 2 C). In the majority (80%) of these specific couples, the MTOC inside the 2H10 cells faced the contact region with the CH12 cell (Fig. 2 A), but the MTOC in the latter remained randomly oriented. Nonspecific couples were formed when the 2H10 cells were mixed with either Ova-pulsed CH12 cells or Cyt-pulsed BCL1 cells, but inside these 2H10 cells the MTOC in ~55% of the cells faced away from the region of contact with the B cell (data not shown), indicating a random MTOC orientation.

These results are closely similar to those previously obtained (1) with the D10 Th cell specific for the Ag Con in the context of Ia^k, and therefore demonstrate the generality of our findings, independent of the phenotype or specificity of the Th cell, or the phenotype of the B cell APC.

Requirement for Antigen Processing by the Ag-presenting Cell. In our previous study (1), the LK B hybridoma cells had been pulsed overnight with the specific Ag Con for presentation to the D10 cells. To determine whether Ag processing might be involved in the specific cell–cell interaction, experiments were carried out in which Con was added to the LK cells just at the time of mixing with the D10 cells, rather than on the previous day. In cell couples formed in such mixtures, the MTOC inside the D10 cells was randomly oriented towards the bound LK cells (data not shown), much as in the case of D10-LK mixtures without any addition of Con (1). This suggested that Ag processing might be required for the specific cell–cell interaction to occur. LK cells were then incubated with Con at 500 µg/ml for 3.5 h with or without 100 µM chloroquine (11, 12). In the absence of chloroquine, such treatment was sufficient to produce cell couples in which the majority (80 ± 5%) of the D10 cells had their MTOC oriented toward the LK cells (Fig. 3, A–C). In the presence of chloroquine, however, cell couples were formed, but in close to 50% of the bound D10 cells, the MTOC was facing away from the LK cells (as in Fig. 3, D–F), corresponding to a random MTOC orientation. As such chloroquine treatment inhibits the endocytosis and processing of antigens, and is thought thereby to inhibit appropriate Ag presentation to Th cells (11, 12; see, however, reference 13), these results indicate that the B hybridoma cell must first process the Ag to induce the specific MTOC orientation inside the Th cell.

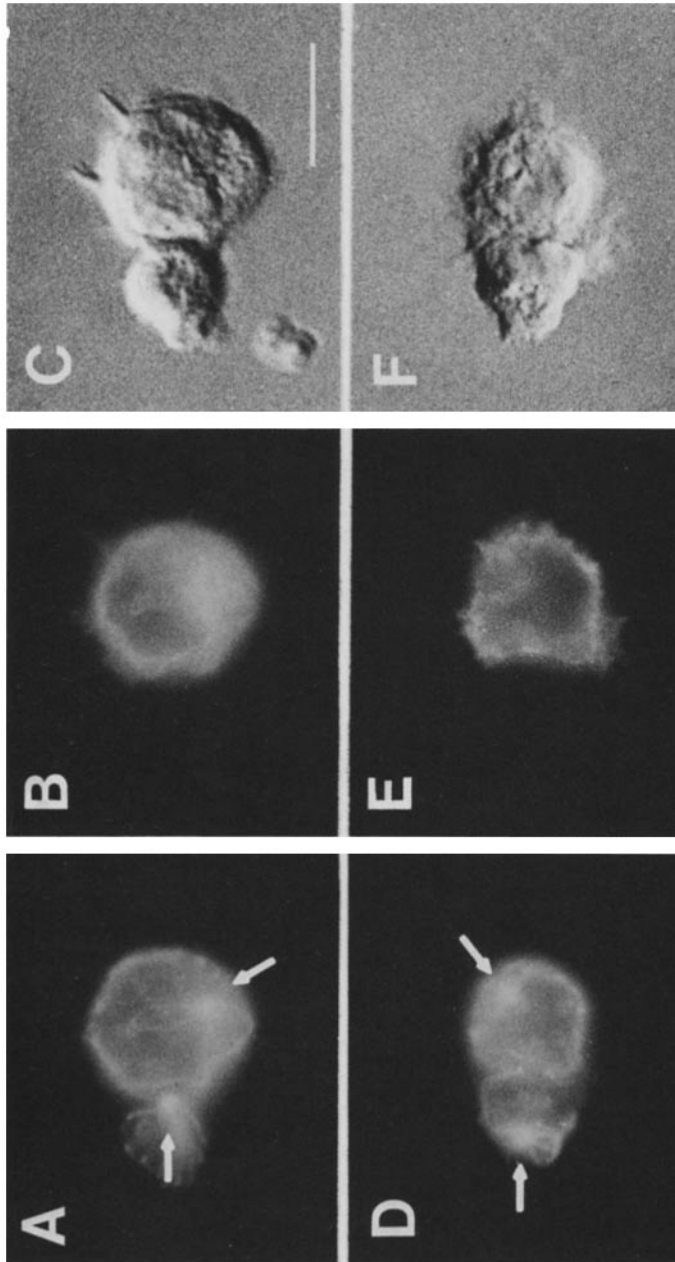*Allogeneic (Ag-independent) Interaction of Th and B Cells.* In the absence of Ag,

FIGURE 1. In this and in Figs. 2–6 each experiment is illustrated by a single cell couple in a row of three panels. The panel on the right is the Nomarski image of the couple; the middle panel involves a cell surface immunofluorescent labeling that always marks the Ag-presenting cell of the couple; while the left panel involves an intracellular immunofluorescent labeling of the same couple for some cytoskeletal component. The details of the latter two immunofluorescent labelings are given in the Materials and Methods section. The bar in the C panels of the figures represents 20 μm; all figures are at the same magnification. (A–C) A D8 cloned Th cell interacting specifically with a CH12 B-lymphoma cell that had first been pulsed overnight with the specific Ag Ova. The B cell is marked for its surface Ig in B. The microtubules and MTOC are immunolabeled in A. The MTOC in the Th cell (horizontal arrow in A) is seen facing towards the bound B cell while the MTOC in the B cell (inclined arrow) is facing away from the T cell. (D–F) Same as A–C, except that the CH12 cell was pulsed with the nonspecific Ag Cyt. Note that morphologically (F) the nonspecific cell–cell couple is not distinguishable from the specific one (C). The MTOC in the Th cell is facing away from the bound B cell (horizontal arrow in D); likewise, the MTOC in the B cell is facing away from the T cell.
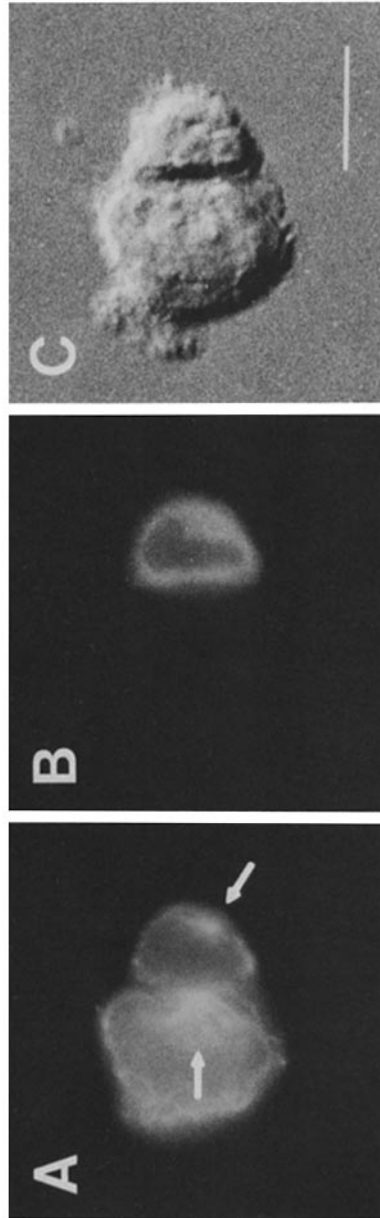
FIGURE 2. A 2H10 hybridoma Th cell interacting specifically with a CH12 B-lymphoma cell that had first been pulsed overnight with the specific Ag Cyt. The B cell is marked for its surface Ig in B. (A) The microtubules and MTOC are immunolabeled. The MTOC in the T cell (*horizontal arrow*) is facing toward the bound B cell, but the MTOC in the B cell (*inclined arrow*) is facing away from the T cell. See Fig. 1 legend for further details.
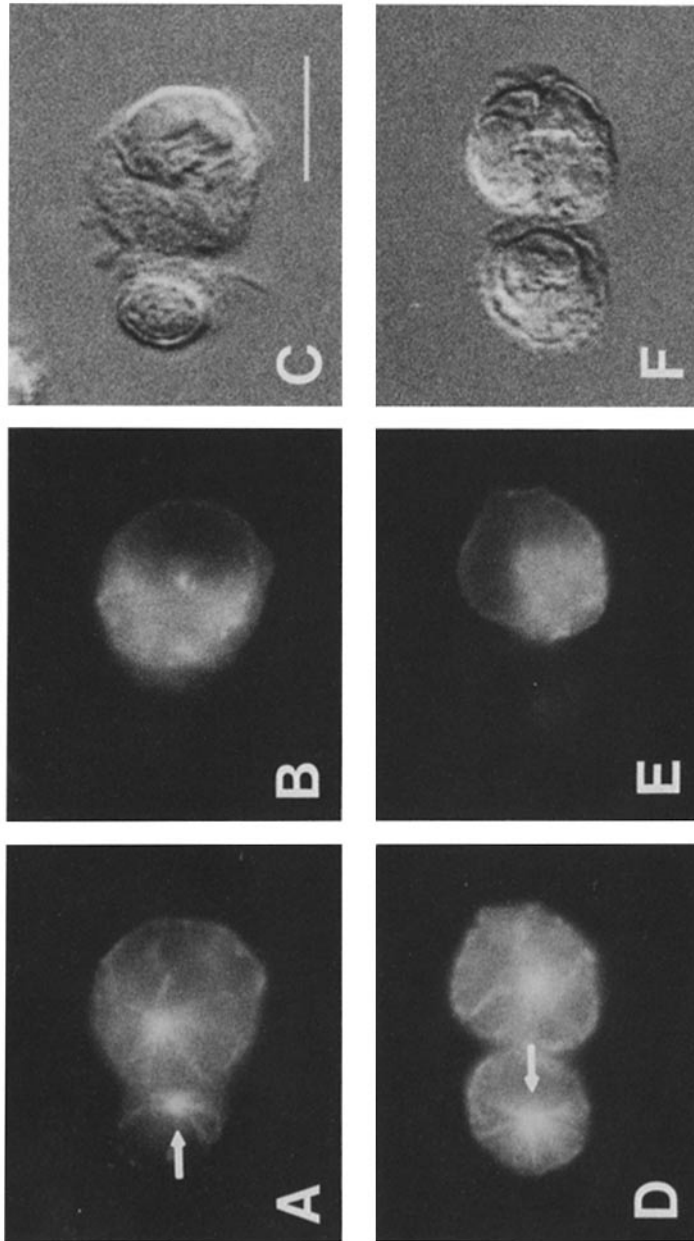
FIGURE 3.   The effects of Ag processing. A D10 cloned Th cell interacting with a LK B-hybridoma cell, that had either been first pulsed with the specific Ag Con for 3.5 h (A–C), or with Con plus 100 μM chloroquine for 3.5 h (D–F). The LK cell is surface immunolabeled for its Ia^d (B and E). The microtu-bules and MTOC are immunolabeled in A and D. The MTOC inside the T cell (arrow in A) is seen to face toward the B cell, but not in the case where chloroquine was present to inhibit Ag processing (arrow in D). See Fig. 1 legend.

D10 cells are known to be activated specifically by allogeneic Ia^b-B cells (6). To investigate this allogeneic interaction, cell conjugates were formed between D10 and LB (Ia^d, Ia^b) cells, were surface immunolabeled with MKD6 mAb (anti-Ia^d) to distinguish the LB cell in the couples, and were then immunolabeled intracellularly to visualize the MTOC. The large majority (95%) of the D10 cells within the D10-LB cell couples had their MTOC facing the bound LB (not shown, but as in Fig. 1A). In cell couples made between D10 and BCL1 (Ia^d) cells, the MTOC inside the D10 cell remained randomly oriented, i.e., 50% of the time facing away from the bound BCL1 cell (not shown, but similar to Fig. 3, D–F). This was also the case with cell couples formed between D10 and LK (Ia^d, Ia^k) cells in the absence of Con (1). Therefore, the allogeneic recognition of class II molecules, as well as the syngeneic recognition of class II molecules plus Ag, leads to a specific MTOC orientation inside the Th cell.

Membrane-associated Cytoskeletal Rearrangements inside Th Cells Bound to Specific APCs. D10 cells were mixed with LK cells that had been pulsed overnight with Con. The cell couples were surface labeled with MKD6 antibody to distinguish the LK cells, and were then immunolabeled intracellularly for one of three different cytoskeletal proteins as described in Materials and Methods. In most of the cell couples examined, talin was found to be clearly concentrated at the contact area between the T and B cells (Fig. 4, A–C). In this figure, a second LK cell appears to be bound to the LK cell that is attached to the Th cell. Talin is not concentrated at this B–B cell junction. Although it is not immediately evident from Fig. 4A, the concentration of talin observed at the D10-LK cell contact region occurred inside the T cell. One demonstration of this is as follows. D10 cells appear to be immunolabeled considerably more intensely for talin than do the LK cells (see Fig. 5A) when the talin in both cells is uniformly dispersed. In Fig. 4A, the similarity of the intensities of talin labeling in the interiors of both the D10 and LK cells therefore indicates that the talin that is concentrated at the cell–cell contact area must be predominantly in the D10 cell, thus depleting the interior labeling of the D10 cell to that seen in the LK cell.

In similar cell couples, in contrast to these results with talin, neither α-actinin (Fig. 4, D–F) nor vinculin (Fig. 4, G–I) showed any significant concentration at D10-LK cell contact regions. The LK–LK cell contacts seen in Fig. 4, D and G also exhibited uniform distributions of α-actinin and vinculin, respectively.

Talin was also concentrated at the cell contacts inside specific cell couples of D8 bound to Ova-pulsed CH12 or 2H10 bound to Cyt-pulsed CH12 (data not shown).

The Concentration of Talin inside the Bound Th Cells Is Antigen- and Ia-specific. Similar cell couples were made between D10 cells and LK cells, but without prior pulsing of the LK cells with the specific Ag Con. Generally, in such couples (Fig. 5, A–C) no marked concentration of talin was observed at the cell–cell contact area such as was seen in the case where the specific Ag was presented on the LK cells (Fig. 4A). In a small fraction (10 ± 5%) of these couples, however, there was a noticeable increase in talin labeling at the cell–cell contact sites (data not shown). In other experiments, BCL1 lymphoma cells (bearing the nonspecific Ia^d determinant) were pulsed overnight with Con, and cell couples were then formed with D10 cells. These couples were surface immunolabeled with mAb
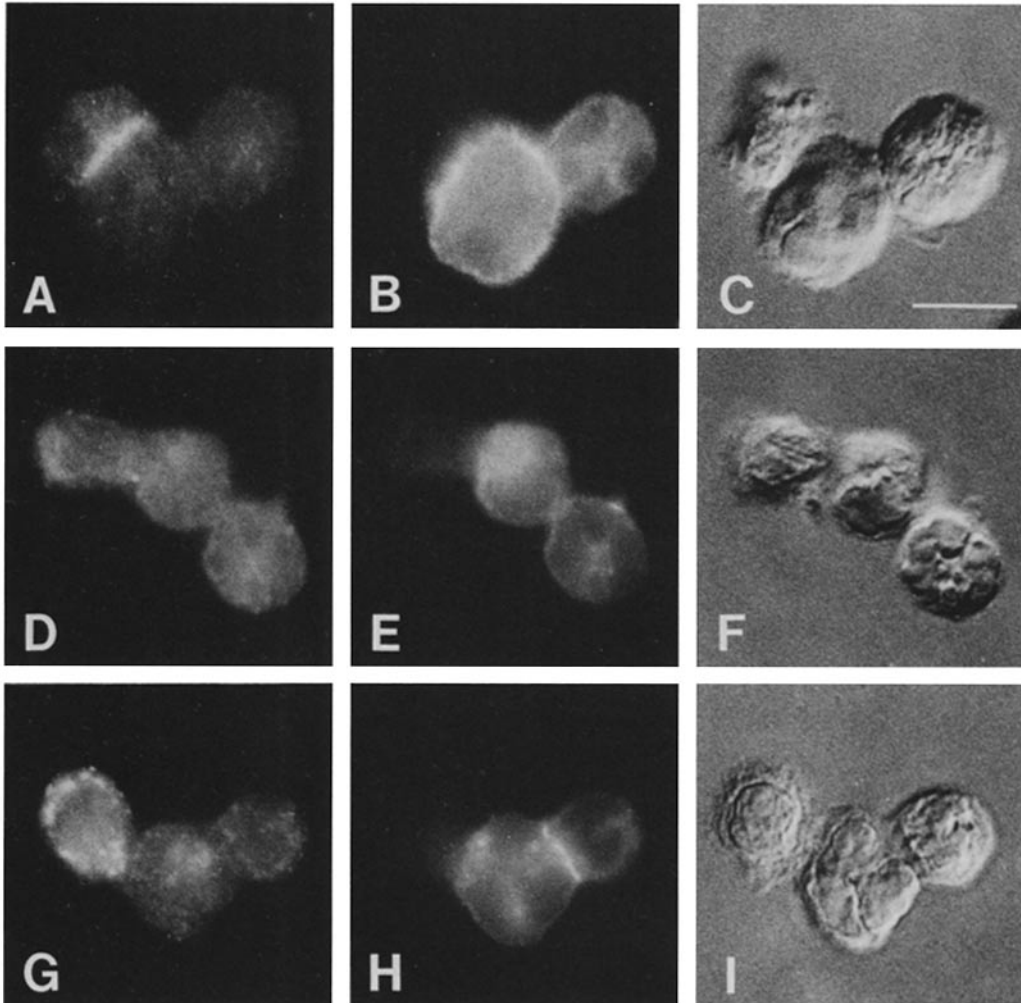
FIGURE 4.   All fields show D10 Th cells interacting specifically with LK cells that had first been pulsed overnight with the specific Ag Con. The LK cells were marked by surface immunolabeling for their Ia^d (*B, E, H*). The conjugates were then intracellularly immunolabeled either for talin (*A*), α-actinin (*D*), or vinculin (*G*). Talin is observed to be concentrated at the contact region between the T and LK cell (*A*), but not where two LK cells are joined. No such concentration is observed in the T-LK contacts with either α-actinin or vinculin. See Fig. 1 legend.

34.5.8 to mark the H-2D^d antigen on the BCL1 cells, and were then immunolabeled intracellularly for talin. The labeling for talin was uniformly dispersed throughout the cells without any consistent indications of a concentration at the cell–cell contacts (Fig. 5, *D–F*).

The concentration of talin inside the Th cell bound to the specific antigen-presenting B cell is therefore dependent on both antigen- and Ia-specific interactions.

The Localization of Talin inside Th Cells Bound to Allogeneic B Cells.   Cell couples formed between D10 and LB cells were surface immunolabeled with
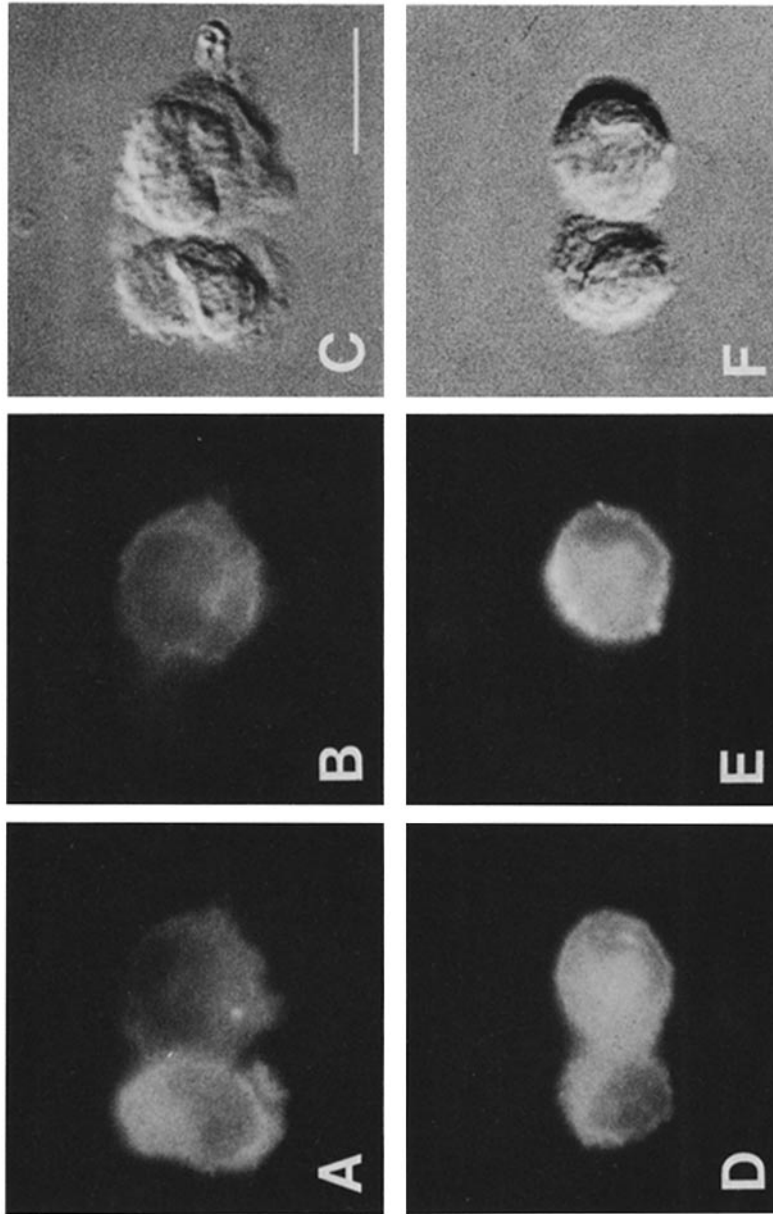
FIGURE 5. The specificity of the talin concentration. D10 Th cells interacting nonspecifically with LK cells in the absence of the specific Ag Con (A–C), or with BCL1 lymphoma cells (bearing the wrong Ia) that had first been pulsed overnight with the specific Ag Con (D–F). The B cells were recognized by surface immunolabeling for their Ia^d (B and E). The intracellular immunolabeling for talin (A and D) showed no detectable concentration of talin at the T–B contact areas. See Fig. 1 legend.

MKD6 antibody and intracellularly labeled for talin. In such couples, talin was generally found to be significantly concentrated at sites of cell–cell contact (data not shown). The intensity of talin labeling at the contact sites, however, was generally less pronounced compared with that observed in cell couples formed between D10 and Con-pulsed LK cells. On the other hand, in cell couples formed between D10 and the nonspecific Ia^d-bearing BCL1 cells, in the absence of Con, the labeling for talin remained uniform throughout the cells (data not shown, but similar to Fig. 5).

The concentration of talin inside the bound Th cell is therefore characteristic of the Ag-independent allogeneic cell–cell interaction as well as the Ag-dependent syngeneic one.

The Effects of Ca^{2+} on the MTOC Orientation and Talin Redistribution within Couples of Th and Antigen-presenting B Cells.   LK cells were pulsed overnight with Con, and presented to D10 cells in a medium that was supplemented with 2.5 mM EGTA and 2.5 mM $MgCl_2$. The cell couples were doubly immunofluorescently labeled with MKD6 and either antibodies to tubulin or to talin. In about 40 ± 5% of the couples, the MTOC in the D10 cell was facing away from the contact area (as in Fig. 6, *A–C*), suggesting a nearly random orientation of the MTOC. However, in another sample of the same couples, talin was generally found to be concentrated at the cell–cell contact sites (Fig. 6, *D–F*). In some experiments, 5 mM $CaCl_2$ was added to the cell mixtures shortly before they were processed for immunolabeling. In these couples, >90% showed the MTOC inside the D10 cell facing the bound Con-pulsed LK cell (Fig. 6, *G–I*). Therefore, extracellular Ca^{2+} is required for the antigen- and Ia-specific MTOC orientation inside the bound Th cell, but not for the concentration of talin at the cell–cell contact site.

The Effects of Ca^{2+} on the Intracellular Events after the Interaction of Th and Allogeneic B Cells.   In a manner similar to the experiments described in the preceding section, cell couples were formed between D10 and LB cells in a medium containing EGTA and $MgCl_2$, and were doubly immunolabeled with MKD6 antibody and with either antitubulin or antitalin antibodies. In 45 ± 5% of the couples, the MTOC inside the D10 cells faced away from the bound LB cells (data not shown), indicating a random orientation of the MTOC. However, in most of the couples, talin was found to be concentrated at the sites of cell–cell contact (data not shown). These results are closely similar to those described in the preceding section, and demonstrate that in the specific allogeneic interaction, as well as in the Ag-dependent syngeneic interaction, the MTOC orientation inside the Th cell is Ca^{2+}-dependent, but the talin redistribution is not.

Discussion

It is well known that CTLs can form stable 1:1 cell couples with their target cells, and that the direct binding of these cells is essential for target cell lysis (reviewed in reference 14). Whether similar cell couples form between Th and B cells has not been clear. The molecular characteristics of Th cell recognition of antigen and MHC on B cells closely parallel those of CTL for their targets; this suggests that Th and B cells also interact directly. Indeed, light microscopic studies have often reported clustering of proliferating and/or antibody-produc-
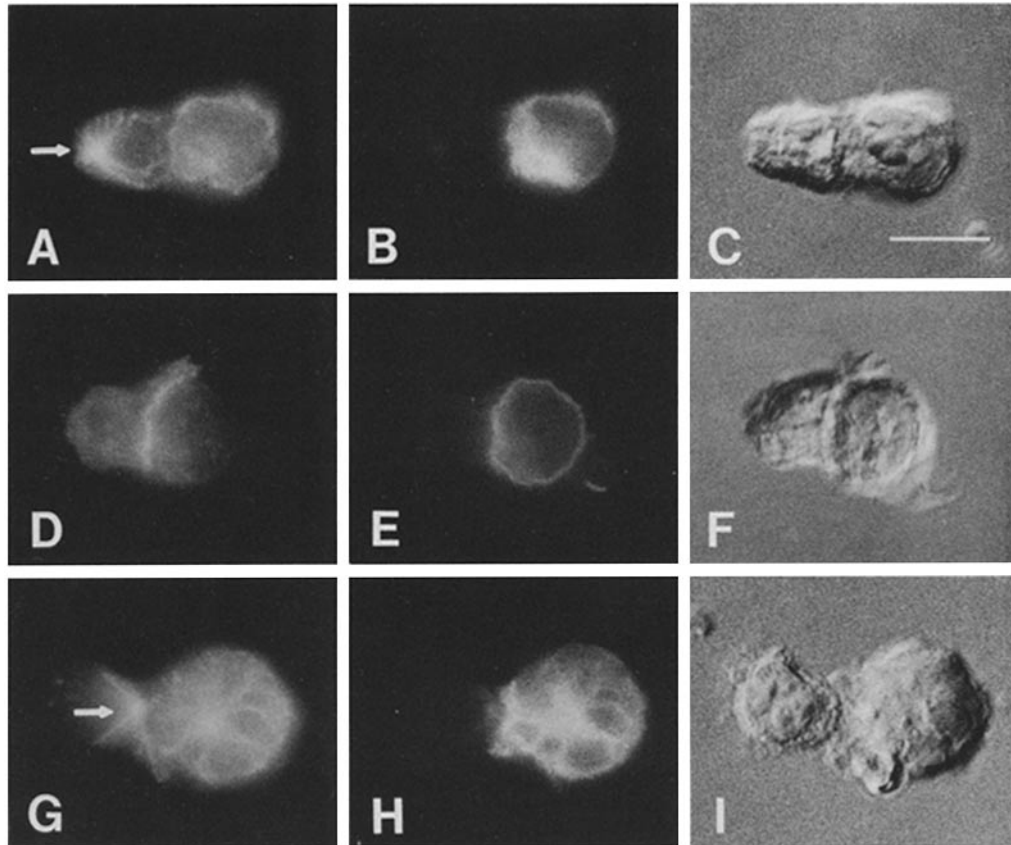
FIGURE 6. The effects of Ca^{2+}. In all fields is a D10 Th cell interacting with a LK cell that had first been pulsed overnight with the specific Ag Con. In all fields, the couples were made in the presence of Ca^{2+}-chelating agents, but in G–I, excess Ca^{2+} was added subsequently. The LK cells were marked by surface immunolabeling for Ia^d (B, E, and H). Intracellular immunolabeling for microtubules/MTOC was performed in A and G, and for talin in D. (A) The MTOC inside the T cell (arrow) is facing away from the bound LK cell when Ca^{2+} was depleted, but faces toward the bound LK cell (arrow in G) when Ca^{2+} was added back in excess. (D) A concentration of talin at the T–LK contact region occurred despite Ca^{2+} depletion. See Fig. 1 legend.

ing lymphocytes with Ag-bearing cells (see references 15, 16). However, the direct 1:1 binding of an Ag- and Ia-specific Th to an Ag-presenting B cell was only recently demonstrated when Ag-specific T cell hybridomas (17) and T cell lines (18) with helper function were incubated with B cell hybridoma or lymphoma cells presenting Ag (1). Because specific and nonspecific cell couples can not be distinguished on light microscopic morphological grounds alone (1), it is essential that criteria be developed to establish that specific interaction has occurred.

Intracellular Criteria for the Direct Specific Interaction of Th with B Cells in Couples Formed Between Them. In the case of couples formed between NK or CTL and their susceptible allogeneic targets, it was previously demonstrated (4, 5, 19, 20) that shortly after cell–cell binding, the MTOC and the GA inside the effector

cell, but not inside the target cell, were coordinately reoriented to face the area of cell–cell contact. Recently, we showed (1) that cell couples could be formed between a cloned Ag- and Ia-specific Th cell and an Ag-pulsed B hybridoma cell, and found that the MTOC inside the bound Th cell was oriented to the site of cell–cell contact. This MTOC orientation was specific for both the antigen and class II MHC recognized by the T cell. Thus, we could visualize the direct interaction of Th and Ag-presenting B cells.

In the present paper, we demonstrate the generality of these findings by showing that a second cloned Th line and a Th hybridoma also engage in specific direct interactions with B hybridomas presenting their respective Ag. In both cases (Figs. 1 and 2, respectively), cell couples formed between the Th cell, and a B cell pulsed overnight with the specific Ag showed an orientation of the MTOC inside the T cell facing the bound Ag-presenting cell.

It must be emphasized that nonspecific couples were formed in all the systems we have so far examined and could not be distinguished from the specific couples on light-microscopic morphological grounds alone (e.g., as observed in Nomarski optics, Fig. 1, C as compared with F). Specific populations of couples could be distinguished, however, by the orientation of the MTOC inside the great majority of specifically bound Th cells.

In the past several years, it has become widely accepted that Ag presentation for recognition by Th cells requires a processing of the native Ag, and the surface expression of the processed components, by the presenting cell (reviewed in reference 12). In the case of a protein Ag, processing apparently involves first the endocytosis of the Ag, followed by its intracellular proteolysis, and finally the appearance of fragments of the Ag at the cell surface of the presenting cell. We have found that Ag must be incubated with the presenting cell >1 h before specfic Th–B interaction involving MTOC reorientation is observed, and that optimum interaction requires at least 4 h (data not shown). This is consistent with a requirement that the antigen be processed by the presenting cell. Furthermore, if chloroquine is added simultaneously with the Ag to the B hybridoma cell, the subsequent formation of couples with the D10 cells does not lead to the MTOC orientation inside the Th cells, whereas in similar experiments carried out in the absence of chloroquine, MTOC orientation occurs (Fig. 3). Although chloroquine may have other effects (13) besides inhibiting endocytosis (11, 12), these observations together support the proposal that Ag processing is required for MTOC orientation. Thus, they further establish that this reorientation is an event that accompanies the significant interaction of Th cells and Ag-presenting cells.

In this paper, a second criterion for a specific direct Th interaction is presented, also based on our previous findings with CTL systems. We demonstrate (Fig. 4, A–C) that a specific concentration of the cytoskeletal protein talin (but not of two other such proteins) occurs inside the D10 Th cell at the region where it is bound to a Con-pulsed, Ia^k-bearing B hybridoma cell, and that this talin redistribution is specific for both Ag and class II MHC. We also found that recognition of specific alloantigen can lead to both MTOC reorientation and talin concentration in the Th cell.

Talin is a 215-kD cytoplasmic protein originally isolated from chicken gizzard

smooth muscle (21). It is localized at several types of cell–substratum and cell–cell adhesion sites (21), and may participate as a peripheral membrane protein in one type of attachment of actin microfilaments to membranes (22). Talin accumulation at the region of cell–cell contact no doubt reflects an extensive and specific type of remodeling of the membrane and the membrane-bound cytoskeleton inside the Th cell since it does not involve a corresponding redistribution of α-actinin or of several other cytoskeletal proteins that interact with F-actin.

The Mechanisms of the Intracellular Reorganizations inside Specifically Bound Th Cells. The rapid, coordinate reorientation of the MTOC and the GA that occurs inside specialized cells that are subjected to a polarized extracellular signal appears to be a fairly widespread mechanism of response. It occurs not only in cell–cell interactions of interest in immunology, involving NK (19), CTL (4, 5, 20), and now Th cells, but also occurs inside motile cells after receiving a polarized signal to migrate (reviewed in reference 3), and inside embryonic epithelial cells upon being stimulated to produce directional secretion of components of a basement membrane (23). It appears likely that the engagement and polarized clustering of certain specific receptor molecules in a cell membrane leads to the reorientation of the GA/MTOC inside that cell to face the clustered receptor. That this behavior is associated with only certain specific cell-surface receptors is demonstrated by the fact that in the cell couples formed between NK, CTL, and Th cells with their specific partner cells, the GA/MTOC reorientation occurs only in the effector cell of the couple.

The GA/MTOC reorientation requires extracellular Ca^{2+}. This has been demonstrated for chemotactic cells in a gradient of chemotactic stimulant (24), for CTL cells bound to a susceptible target cell (4), and, in this paper, for Th cells bound to an antigen-presenting B cell (Fig. 6, A–C). It is possible that the polarized engagement of the specific cell-surface receptor on the effector cell results in the opening of Ca^{2+} channels in the plasma membrane. The influx of Ca^{2+} then leads to some cytoskeletal and/or biochemical changes that bring about the GA/MTOC reorientation. As elevation in cytoplasmic Ca^{2+} has been demonstrated (25) to occur upon antigen-specific Th cell activation. On the other hand, the redistribution of talin that occurs inside the bound Th cell is not dependent on extracellular Ca^{2+} (Fig. 6, D–F).

Possible Functions Served by the Intracellular Reorganizations Inside Specifically Bound Th Cells. It appears likely that the GA/MTOC reorientation that occurs inside bound Th cells, as in the other types of effector cells in which it occurs, serves to direct the traffic of GA-derived secretory vesicles to the region of cell–cell contact. The fusion of such vesicles with the plasma membrane at the contact site may have several consequences (3): (*a*) soluble lymphokines and growth factors that were contained inside the vesicles would be released from the Th cell into the region of cell–cell contact; (*b*) other soluble secretory components, such as cell–cell adhesion factors, proteases, etc., if present, would likewise be released; and (*c*) the membrane mass of the secretory vesicle would be inserted, at least transiently, into the plasma membrane at the contact region (3, 26), and might thereby introduce various integral membrane proteins, such as receptor and adhesive proteins, into the membrane.

Much of the B cell response to Th stimulation is driven by a number of lymphokines secreted by the Th cell upon recognition of Ag on the Ag-presenting B cell. Such lymphokines include an early-acting factor known as B cell stimulating factor 1 (BSF1), previously known as B cell growth factor 1 (BCGF1) (27, 28); a late-acting factor known as BCGFII (29, 30); IL-2, the T cell growth factor that also acts on some B cells (31); and a set of late-acting factors that may be involved in the differentiation to Ig synthesis, known as B cell differentiation factors (BCDFs) (32, 33). The T cell lines used here secrete large amounts of BSF1 and BCGFII (Swain, S. L., unpublished observations). The actions of these lymphokines and growth factors are generally nonspecific; that is, in vitro they can stimulate any B cell carrying the appropriate cell-surface receptors for these factors. It would, therefore, clearly be of great functional importance if in vivo the transmission of these factors was specific, occurring only during direct cell–cell interactions between Ag-specific Th cells and B cells presenting that Ag.

The function served by the concentration of talin inside CTL and Th cells where these cells are in contact with their specific target cell or Ag-presenting cell, respectively, is not clear. One possible function of the membrane remodeling might be to promote a selective fusion of GA-derived vesicles with the contacting membrane of the effector T cell rather than elsewhere on the cytoplasmic surface of the cell.

After this manuscript was completed, we learned of the studies of Sanders et al. (34), who have investigated Th-B cell couples by morphological criteria in light and electron microscopy.

Summary

We have produced and investigated cell couples formed between cloned Th cells or T hybridoma cells, and either Ag-presenting B hybridoma or B lymphoma cells. The specific direct interaction between a Th and B-APC is here demonstrated by two rearrangements occurring inside the bound Th cell; the MTOC (and presumably the GA) is oriented to face the cell contact region with the B cell, and a membrane-associated cytoskeletal protein, talin, becomes concentrated under the contacting Th membrane. In the absence of the specific Ag or the correct Ia determinant, nonspecific T–B cell couples form that are morphologically indistinguishable from specific cell couples in the light microscope, but neither the MTOC nor the talin rearrangement occurs inside the bound T cell of such nonspecific couples. Furthermore, Ag processing by the B cell is required to produce the MTOC and talin rearrangements within the T cell in specific T–B couples. In the case of allogeneic Th–B cell couples, similar specific MTOC and talin rearrangements are observed inside the Th. Extracellular Ca^{2+} is required for the MTOC orientation to occur inside the specifically bound Th cell, but not for the talin rearrangement. It is proposed that the MTOC (and GA) reorientation and the talin rearrangement are involved in the directed secretion of GA-derived lymphokines from the Th cell to the bound B cell.

References

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