Wild type and tailless CD8 display similar interaction with microfilaments during capping

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Summary

We examined the influence of the intracytoplasmic region of CD8α on capping and interaction with microfilaments. We used cell clones obtained by transfecting a CD4+ T-cell hybridoma with (a) T-cell receptor (TCR) α and β chains from a cytolytic clone and (b) CD8α genes that were either native or modified by extensive deletion of the intracytoplasmic region or replacement of the transmembrane and intracytoplasmic domains with those of a class I major histocompatibility complex gene (Letourneur et al. 1990). Double labeling and quantitative image analysis were combined to monitor fluorescence anisotropy and correlation between different markers. Microfilaments displayed maximal polarization within two minutes. The correlation between these structures and surface markers was then maximal and started decreasing, whereas the redistribution of surface markers remained stable or continued. Furthermore, wild type and altered CD8α exhibited similar ability to be capped and to induce co-capping of TCR and MHC (major histocompatibility complex) class I: the fraction of cell surface label redistributed into a localized cap ranged between 40% and 80%. Finally, cytochalasin D dramatically decreased CD8 capping in all tested clones.

It is concluded that the transmembrane and/or intracellular domains of CD8 molecules are able to drive the extensive redistributions of membrane structures and cytoskeletal elements that are triggered by CD8 cross-linking.

Key words: membrane, molecular motion, cytoplasmic domain, cytoskeleton, CD8.

Introduction

Since the elaboration of the fluid mosaic model of the plasma membrane by Singer and Nicolson (1972), it has become clear that many cell functions are affected by lateral movements of membrane molecules. Cell adhesion is thus highly dependent on the lateral mobility of ligand molecules (Rutishauser and Sachs, 1974). Clearly, the formation of strong intercellular bonds may require that ligand molecules be concentrated in adhesion areas (Bell et al. 1990a,b). Different cell surface structures were cross-linked with anti-T-cell receptor, anti-CD8 or anti-class I monoclonal antibodies and anti-immunoglobulin (Fab')₂. Double labeling and quantitative microscopy. Also, the microaggregation of cell surface receptors was examined to monitor the diffusion coefficient of concanavalin A 'receptors' (Schlessinger et al. 1976; Poo et al. 1978; Smith et al. 1979), class I histocompatibility molecules (Edidin and Zuniga, 1984; Bierer et al. 1987; Wier and Edidin, 1986) or lymphocyte surface immunoglobulins (Dragsten et al. 1979; Barisas, 1984) varied between less than 10⁻¹² and about 2×10⁻⁹ cm²s⁻¹. The latter value was roughly consistent with theoretical estimates for free diffusion in a thick viscous sheet (Wiegel, 1984). Thus, it appeared reasonable to view the motion of cell surface proteins as a hindered-diffusion process. In addition, these proteins may undergo active displacements as exemplified by the capping process (Taylor et al. 1971; Bourguignon and Bourguignon, 1984).

Strong experimental evidence suggests that interactions between cytoskeletal elements and membrane intrinsic proteins are dynamic events (Flanagan and Koch, 1978; Burn et al. 1988) that may hinder random diffusion (Koppel et al. 1981; Tank et al. 1982). Further, active movements may be affected by cytoskeletal inhibitors (Taylor et al. 1971; Bourguignon and Bourguignon, 1984). However, the precise molecular interactions involved in these phenomena remain incompletely understood and it is not well known whether cytoskeletal elements are bound to many membrane proteins or...
whether a few membrane molecular species mediate indirect interactions between cytoskeleton and membrane structures, as previously suggested (Bourguignon and Singer, 1977).

Recently, several authors used mutated membrane proteins to assess the influence of cytoplasmic domains on lateral diffusion. Thus, deletion of the intracellular domains of class I histocompatibility molecules (Edelin and Zuniga, 1984), epidermal growth factor receptor (Livneh et al. 1988) or viral proteins (Scuillon et al. 1987) did not change lateral diffusion, whereas a similar mutation resulted in tenfold increase of the diffusion coefficient of MHC class II molecules (Wade et al. 1989).

The present work was planned to evaluate the influence of the cytoplasmic domain of membrane proteins on their active movements and interactions with cytoskeletal elements and membrane proteins. We used a CD4+ T-cell hybridoma transfected with CD8α genes bearing altered cytoplasmic domains (Letourneur et al. 1990). CD8 molecules were capped with specific antibodies and the redistribution of CD8, T-cell receptor (TCR) and MHC class I molecules as well as microfilaments was studied by combination of double labeling and image analysis. It is concluded that the extracellular and/or transmembrane domains of CD8 molecules are sufficient to mediate the extensive interactions observed between these molecules and microfilaments or membrane proteins.

Materials and methods

Cells
KB5C20 (Albert et al. 1982) is an allosreactive H-2Kb-specific cytotoxic T lymphocyte (CTL) clone of B10.BR origin (H-2k). A CD4+ anti-ovalbumin T-cell hybridoma (Marrack et al. 1983) was transfected with α and β chain genes encoding KB5C20 T-cell receptor (Letourneur et al. 1990) and (i) a CD8α gene, yielding DC41.1.4 clone, (ii) a hybrid gene encoding a chimeric polypeptide possessing the extracellular and transmembrane domains of CD8 followed by the last four aminocids from the cytoplasmic domain of the MHC class II Aβ gene product, yielding DC136 clone, and (iii) a hybrid gene encoding a chimeric peptide made of the extracellular domain of CD8 and transmembrane and cytoplasmic domains of class I H-2Kk chain, yielding DC142 clone. The antigenic and functional properties of transfectants were previously described (Letourneur et al. 1990).

Antibodies
Desire is a μ2K mouse anti-clonotypic antibody specific for KB5C20 T-cell receptor (Hua et al. 1996). H59.101.12, a CD8α-specific monoclonal antibody of rat origin (Goldstein et al. 1982), and H84.17.1, a murine μ2K anti-MHC class I H-2Kb monoclonal antibody (Naquet et al. 1985), were kindly donated by Dr M. Pierres (Centre d’Immunologie, Marseille-Luminy). Fluorescein-labeled rabbit Fab’2 anti-mouse immunoglobulin was from Zymed Laboratories (San Francisco) and rhodamine-labeled goat Fab’2 anti-mouse immunoglobulin was from Immunotec (Marseille, France). In some cases, antibodies were biotinylated by exposure to 0.1 mg ml⁻¹ N-hydroxysuccimide biotin (Sigma, St Louis, MO).

Capping and cytoskeleton labeling
Cells were first labeled with monoclonal antibodies at 4°C, then exposed to rhodamine-derived anti-mouse immunoglobulin at 37°C for various periods of time. Finally, they were fixed in the cold with 3.7% paraformaldehyde for 20 min and deposed on glass cover slips. Polymerized actin was then labeled by 20 min exposure to 10 ng ml⁻¹ 1 7-nitrobenz-2-oxa-1,3-diazole phallacidin (NBD–phallacidin, Molecular Probes, Eugene, Or) in pH 7.2 phosphate buffer supplemented with 40 ng ml⁻¹ 1 lysophosphatidylcholine (Barak et al. 1980). Tubulin was stained under similar conditions, using NBD–colcemid (Molecular Probes) instead of NBD–phallacidin.

Immunofluorescence
Our experimental apparatus was described in previous papers (André et al. 1990a,b). Briefly, cells were examined with an Olympus IMT2 inverted microscope, using a 100× dry objective (0.95 numerical aperture) and a 100 W mercury lamp. The image was sent to a single stage intensified Lhese 1036 camera (0.001 lux sensitivity). The video signal was processed with a PCVision+ card (Imaging Technology, Woburn, MA) mounted on a PC20 III Commodore IBM-compatible desk computer. This allowed real-time digitization of the signal, yielding 512×512 pixel images with 256 grey levels. The linearity of light intensity measurements was checked as previously described (André et al. 1990b).

Image analysis
Cells were selected during visible light illumination. Fluorescent images were then digitized and stored on floppy disks for delayed analysis. This was performed as follows: cell contours were obtained by a standard boundary-follow procedure (André et al. 1990b), usually taking as a cutoff value between cell inside and outside, 1.5 times the background level. Since this procedure could not be applied when part of the cell surface was dark after complete capping, cytoskeleton images were used for contour generation.

Cells were then divided into 6 sectors of angle π/3 and the relative fluorescence of each sector was calculated according to the following formula:

\[ R_i^f = \frac{\text{mean sector fluorescence}}{\text{mean cell fluorescence}} \]

where summations are extended over all pixels of the corresponding area, \( f_i \) is the intensity level on pixel \( i \) and \( ff \) is the mean background intensity.

It must be pointed out that relative sector fluorescences are dimensionless parameters that are expected to be independent of cell labeling and detector sensitivity, provided this is linear. The anisotropy of cell fluorescence, which was considered as indicative of capping, was monitored by calculating the standard deviation (S.D.) of the sector fluorescences of all six sectors included in the cell (Fig. 1). As shown in the Appendix, the expected value of this parameter is proportional to the fraction \( x \) of redistributed label on a capped cell, namely:

\[ \text{S.D.} = 1.23 \times x \]

The correlation between both label distributions was also quantified by calculating the correlation coefficient \( r \) between sector fluorescences. As shown in the Appendix, the correlation coefficient obtained on a doubly labeled cell with two independent caps is closely related to the cosine of the angle between the radius vectors of these caps (see also Fig. 5). Since the arithmetic average of a correlation coefficient is meaningless due to the non-Gaussian distribution of this parameter, we used the normalized transform \[ \sqrt{3/2}/(1+(1-r)/(1+r)), \]

the distribution of which is expected to be fairly normal in a random sample (Snedecor and Cochran, 1960). When a single cell is considered, a normalized correlation coefficient higher than approx. 2 may be considered significant with a 95% confidence level.

Evaluation of the importance of potential labeling artifacts
A critical requirement of double labeling experiments was that fluorescent anti-immunoglobulin (Fab’2) bound to a first antibody did not react with the biotinylated second antibody that was subsequently added. This was tested by sequentially exposing DC41.1.4 cells to mouse anti-CD8 antibody, then to fluorescein- or rhodamine-labeled anti-immunoglobulin (Fab’2), then to a biotinylated irrelevant antibody (anti-CD24) of the same class as other biotinylated antibodies, and finally rhodamine- or fluorescein-
derivatized streptavidin. The intensity of biotin-associated labeling was 7% and 6% of that obtained with fluorescent (Fab')2 when both fluorophore combinations (fluorescein/rhodamine and rhodamine/fluorescein) were used.

Although this weak artefactual labeling was inapparent on visual examination of fluorescent cells, it was important to know whether this could significantly alter the correlation between the fluorescence distributions corresponding to different antigens on doubly labeled cells. This was tested by computer simulation: 100 double fluorescence distributions were randomly generated. The mean standard deviation of sector relative fluorescences was 0.28. The mean normalized correlation $(\sqrt{3}/2 \times \ln[(1+r)/(1-r)])$ was then calculated assuming 0%, 5%, 10% and 15% contamination of each fluorescence by the other one. We obtained 0.09, 0.29, 0.51 and 0.78, respectively. It was concluded that the correlation coefficients measured on doubly labeled cells (see Tables) could not be ascribed to artefactual overlap between both labels.

**Flow cytometry**

The mean fluorescence of different populations of labeled cells was determined with a Profile flow cytometer (Coulter, Hialeah, FL).

**Results**

**Tailless CD8 molecules can be capped and interact with microfilaments**

In a first series of experiments, we compared the capping properties of wild-type and engineered CD8 molecules.

Typical fluorescence images are shown in Fig. 1. Cells with limited asymmetry of CD8 are displayed in Fig. 1A,C. A typical redistribution of labeled molecules is shown in Fig. 1E. In this case, sector analysis shows that the mean CD8 fluorescence of the right sector is about 3 times higher than that of left CD8-depleted sectors.

There was a need for quantitative parameters to monitor CD8 capping. The standard deviation of the relative fluorescence of the six sectors defined in tested cells (Fig. 1B,D,F) seemed a suitable choice: this was 0.45 on the capped cell (Fig. 1E,F) as compared with 0.17 and 0.13 of the two other cells (Fig. 1A,C).

The possible occurrence of a relationship between CD8 and microfilament distributions was studied by calculating the correlation coefficient between sector relative fluorescences on doubly labeled cells. This is 0.97 on the cell shown in Fig. 2, which is indeed indicative of a highly significant correlation ($P=0.001$). Since the statistical distribution of the correlation coefficient $r$ in a random population is expected to be strongly non-Gaussian, we used its normalized transform (i.e. $\sqrt{3}/2 \times \ln[(1+r)/(1-r)]$) in order to allow simple averaging of parameters measured on individual cells. This parameter was 3.62 on the cells shown in Fig. 2. A value higher than 2 may be considered as significantly different from zero at the 0.05 significance level.

The kinetics of fluorescence redistribution triggered by cross-linking normal or engineered CD8 molecules is shown in Fig. 3. The anisotropy of CD8 and cytoskeletal fluorescences was nearly maximal within 2–5 min. Wild type and modified CD8 molecules exhibited marked redistribution with comparable kinetics. However, the maximum standard deviation of CD8 relative fluorescences was higher on DC41.1.4 clone bearing native CD8 (0.87±0.06 s.e.) than on DC142 clone with chimeric CD8 (0.61±0.07) and DC136 clone with tailless CD8 (0.52±0.07).

Also, with all tested cells, cross-linking CD8 induced a marked redistribution of cytoskeletal elements, with a maximal correlation between both fluorescence distribu-

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**Fig. 1. Principle of sector analysis.** Cells from the DC 41.1.4 clone were labeled with anti-CD8 monoclonal antibodies, then exposed for 30 min at 4°C (A–C) or 37°C (E–F) to rhodamine-labeled anti-mouse immunoglobulin (Fab')2. Three typical fluorescence images are shown (A, C, E). These images were divided into six sectors of angle $\pi/3$ and the relative fluorescence of each sector is shown (B, D, F). The asymmetry of each fluorescence distribution was quantitated by calculating the standard deviation of sector fluorescences, yielding 0.17 (B), 0.13 (C) and 0.45 (F). The latter value may be considered as indicative of capping. Bar, 5 μm.
Fig. 2. Principle of correlation studies. Cells from the DC41.1.4 clone were labeled with an anti-CD8 monoclonal antibody, then exposed for 30 min at 37°C to rhodamine-labeled anti-immunoglobulin (Fab')2 and fixed before cytoskeleton labeling with NBD-phallacidin. The distribution of CD8 (A and B) and NBD-phallacidin (C and D) was studied by quantitative fluorescence microscopy. Cell images were divided into six sectors of angle $\pi/3$ and the relative fluorescence of each sector was calculated. The asymmetry of each fluorescence distribution was monitored by calculating the standard deviation of sector fluorescences, yielding 0.50 (A,B) and 0.25 (C,D). The correlation between two fluorescence distributions was evaluated by calculating the correlation coefficient $r$ between sector fluorescences and using the normalized coefficient $V^{3/2}\times\ln[(1+r)/(1-r)]$.

The capping of wild type and modified CD8 molecules is dependent on microfilament integrity

It was important to determine to what extent cytoskeletal reorganization was a cause or a consequence of the capping of normal and altered CD8 molecules. This point was addressed by studying the influence of cytochalasin D, a fairly selective inhibitor of actin polymerization (Cooper, 1987), on the capping of CD8. As shown in Table 2, 5 min after the onset of capping, cytochalasin D-treated cells displayed 3–4-fold decrease of the CD8 capping parameter in all tested clones. Thus it was concluded that the cell cytoskeleton plays an active role in CD8 redistribution.

In order to further explore the involvement of microfilaments in the capping process cells were labeled with NBD-phallacidin at various times after the onset of capping. Mean cell fluorescence was assayed with flow...
domains (DC136 clone, C) were exposed to an anti-CD8 monoclonal and anti-immunoglobulin (Fab')2. They were then treated with CD8 modified by replacement of intracytoplasmic and intramembrane residues with those of MHC class I or by extensive deletion of intracytoplasmic clone, A) or CD8 fixed at regular intervals and processed for quantitative analysis of the distribution of CD8 and microfilaments. The standard deviation of relative sector fluorescences of CD8 label (full lines) and microfilament stain (broken lines) is shown on leftwards plots. The normalized correlation between both labels and standard deviations of sector fluorescences were calculated as well as the correlation between both markers. Mean values are shown±standard error of the mean. Number of studied cells in parenthesis.

**Table 1. Absence of substantial redistribution of tubulin molecules after cross-linking CD8**

<table>
<thead>
<tr>
<th>Cell treatment</th>
<th>Control</th>
<th>Ten minute capping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard deviation of sector fluorescence</td>
<td>CD8</td>
<td>0.12±0.014 (9)</td>
</tr>
<tr>
<td>Tubulin</td>
<td>0.06±0.004 (9)</td>
<td>0.10±0.008 (20)</td>
</tr>
<tr>
<td>Correlation between markers</td>
<td>0.66±0.18 (9)</td>
<td>1.1±0.31 (20)</td>
</tr>
</tbody>
</table>

Cells from the DC41.1.4 clone were exposed to anti-CD8 monoclonal antibody and rhodamine-labeled anti-mouse immunoglobulin (Fab')2 at 4°C. They were then fixed immediately (control) or after 10 min incubation at 37°C (capped cells) and stained for tubulin with NBD-phallacidin. The fluorescence distribution was determined for both markers and standard deviations of sector fluorescences were calculated as well as the correlation between both markers. Mean values are shown±standard error of the mean. Number of studied cells in parenthesis.

**Table 2. Effect of cytochalasin D on the redistribution of microfilaments and CD8 induced by CD8 cross-linking**

<table>
<thead>
<tr>
<th>Clone</th>
<th>Cytochalasin D</th>
<th>Phallacidin</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC41.1.4</td>
<td>0</td>
<td>0.19±0.012 (60)</td>
<td>0.80±0.033 (60)</td>
</tr>
<tr>
<td>5 μg/ml</td>
<td>0.18±0.014 (61)</td>
<td>0.56±0.018 (61)</td>
<td></td>
</tr>
<tr>
<td>DC142</td>
<td>0</td>
<td>0.25±0.013 (57)</td>
<td>0.79±0.004 (57)</td>
</tr>
<tr>
<td>5 μg/ml</td>
<td>0.17±0.019 (59)</td>
<td>0.20±0.011 (59)</td>
<td></td>
</tr>
<tr>
<td>DC136</td>
<td>0</td>
<td>0.23±0.018 (37)</td>
<td>0.91±0.056 (37)</td>
</tr>
<tr>
<td>5 μg/ml</td>
<td>0.16±0.016 (38)</td>
<td>0.23±0.019 (38)</td>
<td></td>
</tr>
</tbody>
</table>

Cells from the DC41.1.4 clone (expressing wild-type CD8δ), or DC142 (with hybrid CD8 bearing transmembrane and intracytoplasmic domains of MHC class I molecules) or DC136 (with tailless CD8δ) were exposed to anti-CD8 monoclonal antibodies and fluorescent anti-immunoglobulin (Fab')2, then incubated for 5 min at 37°C with or without 5 μg/ml cytochalasin D. They were then assayed with fluorescence microscopy for quantitative determination of the anisotropy of CD8 and cytoskeleton labeling. Five separate experiments were performed and each value shown as a mean±standard error of the mean. The number of examined cells is shown in parenthesis.

**Table 3. Effect of capping on the microfilament content of DC41.1.4 cells**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean relative fluorescence</td>
<td>1.0</td>
<td>1.02</td>
<td>1.08</td>
<td>1.12</td>
<td>1.13</td>
<td>0.94</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.03</td>
<td>0.03</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Cells were exposed at 4°C to anti-CD8 monoclonal antibodies and anti-mouse immunoglobulins. They were then warmed to 37°C and incubated for different periods of time before being fixed and stained with NBD-phallacidin for determination of total fluorescence with a flow cytometer. Values shown are means of 8 separate experiments.

Cytometry and quantitative fluorescence microscopy. As shown in Table 3, the former method suggested the occurrence of a transient and moderate increase of actin polymerization following capping induction. Fluorescence microscopy did not reveal any modification of phallacidin staining during the studied process (data not shown), due to the lower size of assayed cell populations and subsequent higher dispersion of results.

**Intact and modified CD8 molecules display similar co-redistribution with other cell membrane proteins**

The simplest explanation for the ability of tailless CD8 molecules to interact with cytoskeletal elements was that this association was mediated by other cell membrane molecules that would interact with CD8 through their extracellular or intrabilayer domains and with microfilaments through their intracytoplasmic region in accordance with a model suggested by Bourguignon and Singer (1977). The possibility of extensive interactions between CD8 and other membrane molecules was tested by treating CD8 positive clones with anti-CD8 and studying the extracellular and intrabilayer domains of CD8 and other membrane molecules. As shown in Tables 4 and 5, a close correlation was found between the membrane distribution of CD8, TCR and MHC class I molecules. This suggested the possibility that CD8 might drag cell surface glycoproteins towards a cell pole during the capping process, thus allowing indirect interactions between CD8 and microfilaments.
Capping may involve an early microfilament-dependent and a late microfilament-independent stage.

As shown in Fig. 3A,B,C, the correlation between cytoskeleton and CD8 distributions was maximum within about 2 min after exposure to the capping stimulus. This correlation then decreased, whereas the anisotropy of fluorescence distributions kept on increasing. This suggested that CD8 capping involved some kind of association between extra- and intra-cellular structures only during the earliest phase of the capping process. Indeed, 10–30 min after the triggering of redistribution, we observed cells displaying redistributed CD8 and microfilament markers, with a different intracellular localization (Fig. 4). It was of interest to know whether these findings applied to other cell surface markers, in addition to CD8. As shown in Fig. 5, when anti-TCR or anti-MHC class I antibodies were used to induce capping, a co-redistribution of cell surface markers and microfilaments was also found, and the correlation between extra- and intra-cellular markers was maximum within two minutes, then decreased, as was found with CD8.

**Table 4. Co-capping of MHC class I molecules by anti-CD8 antibodies**

<table>
<thead>
<tr>
<th>Clone</th>
<th>Standard deviation of sector fluorescence</th>
<th>Correlation between markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC41.1.4(n=23)</td>
<td>1.02±0.07</td>
<td>0.34±0.03</td>
</tr>
<tr>
<td>DC136(n=14)</td>
<td>0.58±0.09</td>
<td>0.23±0.04</td>
</tr>
</tbody>
</table>

Cells were treated with anti-CD8 monoclonal antibodies and exposed for 20 min at 37°C to fluorescein-conjugated anti-immunoglobulin Fab'2. They were then cooled and labeled with rhodamine-derivatized streptavidin. They were then examined with quantitative fluorescence microscopy for determination of fluorescence asymmetry (as expressed by the standard deviation of sector fluorescence) and correlation between both antigens (as expressed with normalized correlation). Mean values are shown ± standard error. Number of analyzed cells in parenthesis.

**Table 5. Co-capping of T cell receptor by anti-CD8 antibodies**

<table>
<thead>
<tr>
<th>Clone</th>
<th>Standard deviation of sector fluorescence</th>
<th>Correlation between markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC41.1.4(n=20)</td>
<td>0.60±0.06</td>
<td>0.58±0.05</td>
</tr>
<tr>
<td>DC136(n=14)</td>
<td>0.76±0.04</td>
<td>0.34±0.02</td>
</tr>
</tbody>
</table>

Cells were treated with anti-CD8 monoclonal antibodies and exposed for 20 min at 37°C to fluorescein-conjugated anti-immunoglobulin Fab'2. They were then cooled and labeled with rhodamine-conjugated streptavidin. They were then examined with quantitative fluorescence microscopy for determination of fluorescence asymmetry (as expressed by the standard deviation of sector fluorescence) and correlation between both antigens (as expressed with normalized correlation). Mean values are shown ± standard error. Number of analyzed cells in parenthesis.

**Fig. 5. Capping of TCR and MHC class I.** Cells from the DC41.1.4 clone were exposed to an anti-TCR (A) or anti-MHC class I (B) monoclonal antibody and anti-immunoglobulin Fab'2. They were incubated at 37°C for various periods of time, then processed for quantitative analysis of the distribution of TCR or MHC and polymerized actin. The standard deviation of relative sector fluorescences for TCR or MHC (full lines) or microfilament (broken lines) is shown on the right-hand plots. Each point represents a mean of 20–30 determinations. Vertical bar length is twice the standard error.

A first requirement was to achieve a quantitative description of the tested phenomenon. The sector analysis we performed was felt a satisfactory way of monitoring the capping process since in most cases the value of the standard deviation parameter matched the intuitive feeling of the extent of antigen redistribution provided by visual examination of fluorescence images (Fig. 1). A second conclusion made clear by quantitative fluorescence determinations is that only a limited fraction of cross-linked cell surface molecules were capped. Indeed, using the derivation shown in the Appendix, the fraction of capped molecules was found to range between 40% and 80% of total label. Obviously, it is difficult to evaluate fluorescence intensities using a grey scale (Figs 1, 2). As shown in Fig. 4, a coded color display gives a much more precise description of the distributions of light intensities.

Now, the most clearcut conclusion of our experiments is that wild type and altered CD8 chains were all able to be redistributed in response to a cross-linking stimulus, and they induced similar redistribution of polymerized actin (Fig. 3). The simplest interpretation for these findings is that the redistribution of cytoskeletal elements as well as TCR or MHC class I molecules induced by bridging ‘tailless’ CD8 on DC136 clone was mediated by molecular interactions occurring in the bilayer or the extracellular region. This might involve some third-party structure, in accordance with a model previously reported by Bourguignon and Singer (1977). Direct CD8–cytoskeleton interaction did not play a quantitatively significant role in this respect. This conclusion is consistent with the finding that

**Discussion**

The purpose of our work was to assess the role of the intracellular domains of membrane molecules on the active movements of these structures. Capping was chosen as a suitable model for this study.
Fig. 4. Coded color display of immunofluorescence distributions. Cells from the CD41.1.4 clone were exposed to anti-CD8 monoclonal antibodies and anti-immunoglobulin (Fab')_2 for 5 min (A and B) or 30 min (C and D) at 37°C before fixation and staining for quantitative analysis of the distribution of CD8 (A and C) and polymerized actin (B and D). Fluorescence intensities were represented with coded color display using a 16-level scale. The normalized correlation between both fluorescence distributions is 0.81 (A and B) and -0.42 (C and D).
complement decay-accelerating factor, a lipid-anchored membrane protein, could be made to cap and interact with cytoskeletal elements as efficiently as CD3, CD4 or CD8 (Kammer et al. 1988). It must be emphasized that specific intermolecular associations may not be required to mediate interactions between microfilaments and cell surface proteins, since a bulk displacement of a given molecule may generate a lipid flow in the viscous bilayer or drive extracellular glycoalyx elements entangled with the oligosaccharide chains of moving molecules. Another finding is that capping CD8 molecules induced a significant co-redistribution of TCR (Table 5) and, to a lesser extent, MHC class I (Table 4) molecules. It must be pointed out that this co-redistribution was sometimes limited (see 1st row of Table 4 and 2nd row of Table 5). Perhaps co-capping is not an all-or-none phenomenon, as pointed out that this co-redistribution was sometimes limited (Andre et al. 1990a, 1990b). geometrical model of fluorescence images to calculate the average value of $f$. We found:

$$f = 1.23 \pm 0.58 \text{ (standard deviation).}$$

Significance of correlation

The significance of the correlation between fluorescence distributions was assessed with a similar procedure to that performed for the standard deviation. Since the correlation coefficient between two random variables $x$ and $y$ is the same as that between $ax+b$ and $cy+d$, where $a$, $b$, $c$ and $d$ are real constants, the correlation between the fluorescence distributions of two spheres with a combination of uniform label and a point fluorescence (as described above) is only dependent on the location of both concentrations of fluorescent points. Thus, we considered 4800 points

$$\sum_{i=1}^{6} p_i = 1.$$
Fluorescence concentrations were generated for determination of the directions of fluorescent points. Results are shown for 10 classes of cosine values (vertical bar length is twice the standard deviation of correlation parameter).

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