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FRANK J. DIXON HENRY G. KUNKEL

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PREFACE

The subjects presented in this volume emphasize the breadth of immunology in the areas of science to which it contributes and from which it draws information and technology. In this interdisciplinary exchange, it is becoming apparent that the cellular and humoral events involved in immunologic reactions are similar in many ways to their counterparts in other systems. The lymphocyte has become one of the most successfully exploited subjects of cell biology and that exploitation has ultimately shed light on immunologic processes. Consideration of the part played by interactions between the plasma membrane and cell cortex in lymphocyte function brings the cellular events of immune responses within the framework of cellular activities in general. Only recently have the elaborate, multiple control mechanisms governing immunologic responses begun to be appreciated, and these also seem to fit within the general pattern of controls operating elsewhere. Analysis of controls operating in the contact sensitivity response indicates that they include the same elements, with perhaps some variation in emphasis, that operate in other immune responses. The discussion of self-tolerance and possible reasons for its breakdown with consequent development of autoimmunity also deals with the same regulatory processes that monitor usual immune responses. Even the manifestations of autoimmune disease are produced by a perversion of ordinarily protective mechanisms operating either singly or in combination. The description of host–viral relationships in LCM infections indicates the complexity of mechanisms related both to viral propagation and to host defense which interact to determine the nature of the largely immunologic disease that results.

Surfaces of lymphocytes and macrophages in large part determine and control the multiplicity of cellular interactions that characterize immune function. The organization and physiology of cell surfaces are in turn determined not only by intrinsic plasma membrane markers and receptors but also by the state of the underlying cell cortex which contains the cytoskeleton and cytomusculature. This critical interrelationship between cell cortex and plasma membrane and its role in determining immunologic function are reviewed in the first chapter of this volume by Dr. Francis Loor. Two general kinds of plasma membrane interactions are considered in detail. The first is an active process of motion and/or recognition initiated in the cortex and usually involving formation of uropods and microvilli where receptors are

concentrated. The second is passive recognition in which resting lymphocytes are exposed to ligands that bind to and cluster receptors thereby perturbing the cells' plasma membranes and initiating the biochemical events of activation.

In the second chapter Drs. Claman, Miller, Conlon, and Moorhead discuss controls operative in the expression of experimental contact sensitivity, drawing on their own considerable work in this field. The events leading to contact sensitivity including the essential reactivity of the antigen with self molecules, the involvement of Langerhans cells of the skin, and the eventual development of effector T lymphocytes are clearly presented. This chapter then focuses on several mechanisms influencing the extent of a sensitivity response. Controlling factors include route and amount of antigen presentation, which to a great degree determine whether sensitization or tolerance will develop, production of soluble suppressor substances that may accompany tolerogenic regimes of antigen administration, formation of inhibitory antiidiotypic antibodies, and development of T suppressor cells. These multiple regulatory mechanisms operating in contact sensitivity are quite similar to those involved in other cellular immune responses as well as in humoral responses and are in keeping with the general elaborate regulation controlling immunity.

The relationship between the normal state of self-tolerance and its abnormal corollary, autoimmunity, is presented in the third chapter by Dr. Weigle, a long time authority on this subject. The several possible bases for loss of self-tolerance, including failure of immune regulation and abnormal presentation of potential self-antigens, are considered in terms of current understanding of cellular and humoral immune processes. A particularly thorough evaluation of suppressor cells and their possible role in self-tolerance and autoimmunity is given. Finally, the pathogenic mechanisms that may be involved in autoimmune disease are examined in the light of two familiar experimental models, allergic encephalomyelitis and autoimmune thyroiditis. The pathogenic potential of cellular autoimmune responses in encephalomyelitis, on the one hand, and of autoantibody responses in thyroiditis, on the other, indicate the very different pathogeneses which may operate in autoimmunity.

Study of LCM viral infections has led to a surprising number of conceptual immunologic advances. It was initially postulated that infection occurring prior to maturation of the immunologic system might culminate in tolerance to the infecting virus via clonal elimination of responsive lymphocytes—a concept later extended to account for normal self tolerance. Eventually, research showed that complete im-

munologic tolerance could not be induced by viral infections initiated at any time and that the diseases accompanying LCM infection were caused by host–T cell responses in acute infection and antibody–LCM virus immune complexes in chronic infections. In the fourth chapter Drs. Buchmeier, Welsh, Dutko, and Oldstone, who have contributed greatly to this subject, present in molecular terms the explanation for immunologic events accompanying LCM infection, including the dual recognition of viral and histocompatibility antigens essential for T cell action and the modulation of viral expression resulting from the immune reponse. Finally, they discuss the important but less well appreciated aspects of nonimmunologic regulation of viral infection particularly the role of defective interfering virus.

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FRANK J. DIXON
HENRY G. KUNKEL

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ADVANCES IN IMMUNOLOGY, VOL. 30

Plasma Membrane and Cell Cortex Interactions in Lymphocyte Functions

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I. Introduction

Structural and dynamic aspects of the lymphocyte surface physiology have been the subject of a few recent extensive reviews (125, 363, 438, 439, 570). During the last few years a number of observations have shed new light on our comprehension of the phenomena. Various structures in the lymphocyte cortex influence the organization of the lymphocyte surface, and this is especially evident for the expression of microvilli (MV), the formation of the cap and the uropode, and gross cell locomotion. The influence of the lymphocyte cortex can be de-

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duced from the effects of drugs known to interfere with its activity, from observations with the electron and fluorescence microscopes, and more recently from the biochemical analysis of lymphocyte surface components.

Here I will attempt to summarize the most critical progress made in the understanding of the organization of the plasma membrane and of the cell cortex. This will be developed *in extenso* elsewhere, together with the building of a hypothetical model explaining how interactions of plasma membrane components and cell cortex elements may result in the gross morphological changes frequently associated with cell activation (367). My purpose here is to review the experimental evidence showing that both the plasma membrane organization and the activity of the cell cortex elements are implicated in the immunological function of the lymphoid cells.

II. Structural Relationships of the Plasma Membrane (PM) with the Cell Cortex

All eukaryotic cells seem to contain filaments and tubules, i.e., a cytomusculature (Cm) and a cytoskeleton (Cs). Thin microfilaments (MF, 5–8 nm diameter, made of actin) and microtubules (MT, 24 nm outside diameter, made of tubulin) are regularly detected, but thick filaments (13–25 nm diameter, made of myosin) usually are not, and intermediate filaments (10 nm diameter) are as yet not enough studied to decide their ubiquitous distribution (196). Even if all Cs and Cm elements were present in all types of eukaryotic cells, there could be important differences in both their content and their organization. As an example, actin represents up to 20–30% of the protein content of mobile cells, but only 1–2% in some tissue cells (327). Furthermore, even though polymorphism of the two major proteins, actin and tubulin, is limited, there can be important heterogeneity, even within a single cell, of the content, distribution and organization of actin-made MF and tubulin-made MT. For instance, in fibroblasts, two distinct sets of MF seem to exist, which have different properties and assume different functions; similarly, not all the MT of a cell show the same sensitivity to a drug-induced depolymerization (363, 364). The cell has indeed multiple tools to control the state of assembly and the activity of such structures. Among these are, in the case of MF, the myosins, which are more polymorphic (663), and the accessory proteins such as the tropomyosin, the troponins, the filamin, the profilin (72), and in the case of MT, the not yet well defined microtubule associated-proteins (MAPs) found along MT (595, 596). If, in the cell, MT and MF show some physical association, such an interaction

would probably be regulated by the accessory proteins. At least, *in vitro*, there is evidence for it (222). It is, however, still difficult to integrate the presently known data (for review, see 367) into a general model of cell motility, and to understand fully those activities of lymphocytes that depend on various kinds of membrane movements. Particularly, the information available for lymphocytes and, more generally, leukocytes is still limited.

In mobile eukaryotic cells, the principal constituent of MF, actin may represent 10–30% of the total protein of the cell (327). Actin is a major component of the normal lymphocyte (27); it represents some 6% of the total protein of a guinea pig B cell leukemia line (160). Actin is usually a major component of purified PM of lymphocytes (26), and the amount of PM-bound actin may represent some 10% of the total cellular actin (168). Myosin can represent up to some 5% of the total protein of nonmuscle cells (468). In polymorphonuclear leukocytes, a value of 2.4% of total protein has been reported (623). To my knowledge, there are no data for lymphocytes in general, but in a particular type of lymphocyte (acute lymphoblastic leukemia) it represents only some 0.63% of the total cell protein (468), and in another type (B cell leukemia cell line) it represents from 0.68% to 0.83% of total cell protein (160). Other proteins, such as α-actinin, tropomyosin, and troponins (having structural and regulatory functions in muscular fiber contraction), have not been quantitatively determined in nonmuscle cells, but some of them have been detected in lymphocytes. As far as MT are concerned, their major constituting proteins, the tubulins, represent 0.5% to a maximum of 1.0% of the total cell protein (594). The possible association of tubulin with the PM is still controversial (226). The high molecular weight proteins associated with MT have been detected in fibroblasts (596).

Recently developed methods of immunofluorescence (IFM) have allowed the detection of some of those Cs/Cm proteins in lymphocytes, although most extensive studies have so far been performed with fibroblasts, epithelial cells, macrophages, polymorphonuclear neutrophils, etc. (e.g., 203). The methods make use of various specific antibodies (anti-actin, anti-tubulin, and so on) that may show different specificities from batch to batch, thus possibly explaining some discrepancies. Two disadvantages of these methods are their relative lack of sensitivity when directly labeled antibodies are used (direct IFM), and the difficulty of trying to look simultaneously at two different Cs/Cm and membrane components when indirect IFM is used. Moreover, although there is more actin than myosin in the cells, the specific antibodies usually give rather poor detection of actin (see 150, 154),

compared to an effective detection of myosin, which may be due to a difference of immunogenicity, actin being more evolutionarily conserved than myosin. An elegant nonantibody method can be used to detect actin: it consists of detecting actin through the binding of biotinylated heavy meromyosin, which is itself revealed by binding of fluoresceinated avidin (which binds avidly to biotin) (254). This allows great sensitivity of actin detection, possibly simultaneously with detection of another Cs/Cm or PM component by sensitive indirect IFM. The Cs/Cm elements can be detected only after fixation and lipid extraction to make the membrane permeable for the reagents, but no binding is detected on viable cells, showing that no antigenic determinants specific to Cs/Cm components are exposed on the cell surface. There are some controversial cases, however, that were reported for myosin exposed on the outside of the cell membrane (discussed in 327). In at least one case, the cell surface component that was detected by some of the antibodies in the anti-myosin serum appeared, however, to be unrelated to myosin (142).

In resting, rounded lymphocytes, all Cs/Cm components that have been detected so far (actin, α-actinin, myosin, and tubulin) were distributed homogeneously as a faint rim at the periphery of the cell (142, 693). Thus, in contrast to the spectacular IFM detection of Cs/Cm components in the flat interphase fibroblast, one does not find in resting lymphocytes splendid microtubular networks or microfilamentous stress fibers. However, when the interphase fibroblast is rounded, most of the IFM staining that detects MF and MT is then diffuse, with some concentrated at the periphery, near the surface in the cortical cytoplasm of the cell (184). Resting lymphocytes are thus similar to rounded interphase fibroblasts in that respect.

Alterations of such homogeneous distribution of Cs/Cm proteins in the lymphocyte cortical cytoplasm can be observed under two main circumstances: (*a*) when the lymphocyte develops long microvilli (MV), either spontaneously or induced, actin is found within the MV (151–153, 693–695); and (*b*) when the lymphocyte shows a uropode, either spontaneously or induced, actin is found to be more concentrated within the uropode, together with myosin and tubulin (572, 634) (see Sections III and IV).

Before IFM methods became popular for the detection of Cs/Cm proteins, electron microscope methods had already shown the way. Thin MF filaments 4–6 nm in diameter had regularly been observed under the PM of lymphocytes, forming a kind of narrow, dense network (124). Such thin MF are likely to be made of actin. Thick bundles of MF were also found within MV and pseudopode-like structures of

lymphocytes (692). Early studies on capping had also indicated the particularly high concentration of MF in the tail of lymphocytes capped with anti-Ig antibodies (510). Thick filaments made of myosin have not been reported to occur in lymphocytes, but a few filaments 15 nm thick and 300 nm long were observed in glycerinated horse neutrophil leukocytes (587), and similar filaments form *in vitro* from the myosin extracted from a B cell leukemia line (160). Lymphocytes at 37°C have numerous MT assembled, most or perhaps all of which would be connected to the centrioles (125). Apart from these, the distribution of the few MT in the cytoplasm of lymphocytes appears to be random, rather than specifically associated with the membrane (125, 692). Although some MT have been observed running under the PM (124, 692), they remain separated from it by the cortical layer of MF (124). With the exception of MT, which are found in the uropode, MT do not seem to be associated with the PM (124). Rare MT were observed to extend perpendicularly to the PM (125, 692). The total number of MT that terminate after the PM in a cell was estimated to be probably one hundred at most (125). Their presence within "microspikes" (629) or within each MV present on the uropode of the motile lymphocytes (399) had been reported in the past, but the nature of these structures (MT or MF) remains controversial. Treatments or drugs known to affect MT integrity do not interfere with the expression of lymphocyte surface structures such as MV (368, 444, 445). If anything, drugs like colchicine and vinblastine actually increase the development of a uropode and the extent of capping. Conversely, the various cytochalasins (A–E), which inhibit MF function in different ways, can abolish the emergence of MV on lymphocytes (as well as on other cells) (127, 152, 368, 444, 445) and they can inhibit the capping in varying degrees (123, 124, 361, 362, 368, 371, 639), cytochalasin A in my experience showing most inhibition, and cytochalasin C showing the least (F. Loor and L. Ångman, 1980, *Exp. Cell Res.*, in press).

III. Uropode Formation and Capping.

The general characteristics of the capping phenomenon, especially its requirement for energy, its inhibition by low temperature, its inhibition and its reversal by cytochalasin, and the accumulation of MF under the cap, have led to the suggestion that capping is probably a contractile phenomenon: it would be mediated by contractile MF bringing patches of aggregated membrane components to an area of the cell where they would be endocytosed and digested, or be shed (54, 55, 125, 363, 365, 371, 438, 570). The role of MT appears to be

more complex. Apparently, the motility of MF would not be synergized, but rather antagonized, by MT, whose role is to establish cell shape and to maintain its compartmentalization. Evidence for the inhibitory role of MT comes essentially from extensive experience with concanavalin A (Con A) capping. In some circumstances (reviewed in 125, 363, 438, 439, 570), Con A does not cap; on the contrary, it even inhibits capping of other cell surface macromolecules by their own antibodies. Such inhibition is abolished by treatments that depolymerize MT (141, 362, 368, 374, 646, 691, 692). Along the same lines, mIg or Con A sites cap slowly on CBA mouse lymphocytes and rapidly on A mouse lymphocytes, but after colchicine treatment, cells from both mouse strains show fast capping (173). In the Beige mouse strain, fast capping of Con A on polymorphonuclear leukocytes occurs in absence of drugs that depolymerize MT (457). That this were due to a genetic defect of MT polymerization (457) has been questioned (174), but it remains possible that some MT only present anomalies of polymerization or that the association of MT with the PM is altered in Beige mouse leukocytes. A synergy for capping inhibition was obtained by simultaneous use of colchicine or vinblastine with cytochalasin (123, 124, 646). In view of more recent data reviewed below, this phenomenon probably results from MT disruption that provokes a complete reorganization of the Cm leaving in the cell cortex surrounding the nucleus much less MF, which would be readily inhibited by cytochalasin. A clue for understanding whether MT antagonize or synergize with MF for capping could be found *a priori* in their overall structural location in the cell. As indicated above, IFM detection of MT and MF does not help that purpose very much. In the flat interphase fibroblasts, such methods indicate that MT and MF have a different distribution; i.e., most of them are not localized in the same parts of the cells and, *a fortiori*, not in the same parallel fiber system or other kind of network (184, 255). However, their organization in rounded fibroblasts is not known and may eventually be revealed to show interactions.

Recently a number of researchers have looked for the possible existence of transmembrane linkages between Cs/Cm elements and PM components exposed on the outside. These links may occur either directly between a transmembrane glycoprotein [such as HLA (656)] and the Cs/Cm elements, or indirectly via other integral membrane proteins if the PM component exposed at the outer cell surface does not cross the membrane [such as mIg (656)], or indirectly via Cs/Cm-associated elements, such as the α-actinin molecules associated with MF or perhaps the MAPs (MT-associated proteins), but this is purely

speculative. The first attempts of this kind did not show any concomitant redistribution of α-actinin, of cytoplasmic myosin, and of actin in lymphocytes when capping of H-2, Con A binding sites, or mIg was induced on the cell surface (142). However, there is now a substantial amount of evidence to the contrary. mIg capping on mouse B cells cocaps tubulin and actin, which are now found under the mIg cap in the cytoplasm of the uropode (185), a similar result being obtained when myosin is detected (572). Cocapping of Con A and actin has also been observed for fibroblasts (634), for rabbit neutrophils (459), and for human leukocytes (6).

Immediately the question arises whether cocapping actually demonstrates a linkage of the surface components with the Cs/Cm structures that are coredistributed in the uropode, or whether it has some trivial explanation, e.g., that capping stimulates uropode formation. Whichever is the case, most of the lymphocyte cytoplasm flows from its thin rim distribution around the nucleus to the uropode, bringing with it all cell organelles, including Cs/Cm proteins. The fact is that a single capping process seems to bring all the actin, myosin, and tubulin that is detectable by IFM into the cap/uropode area, with practically no Cs/Cm proteins detectably left in the rest of the cell. Hence, one can induce a lymphocyte to make two or three successive cappings of PM components at short time intervals. This may be due to recruitment, to rapid recycling of the myosin. Moreover, the number of cells showing cocapping of myosin and mIg consistently represents only 70–80% of all cells showing mIg capping (572). Thus, there can be capping of PM components without detectable coredistribution of Cs/Cm components (142, 572). Inversely, there are always a proportion of cells spontaneously forming a uropode where Cs/Cm proteins detectable by IFM accumulate (6–12% of T cells) (572).

Capping of Con A on leukocytes (depleted from MT by drugs) occurs at one cell pole on a protuberance that consists of highly plicated membrane sustained by a network of densely packed MF, each plication containing a bundle of MF (6). The formation of such a structure (which I would call a uropode studded with microvilli) is not *induced* by the Con A, but simply follows the disassembly of MT per se. The migration of MF does not bring detectably more membrane Con A binding sites on the uropode, and cells having a preformed uropode can still redistribute their Con A sites initially dispersed on the whole cell (6). This shows that the bulk of MF or MT found in the uropode is not involved, or at least is not needed for dragging liganded membrane components to the cap. This provides a possible explanation for the synergy between colchicine and cytochalasin for capping inhibition:

in presence of colchicine, only few MF are left in the cortical cytoplasm of the cell, and the effects of cytochalasin can be more dramatic.

On T cells, not only the capping of two different PM components (H-2 and T 25) coincides with the redistribution of myosin and actin as subcaps, but there is also a coincidence at the earlier stage of patching: the intracellular PM-associated actin or myosin becomes clustered into submembranous patches located directly under the external PM component patches (54, 55). Similar results are obtained for the patching and capping of Con A on HeLa cells; these cells are larger than lymphocytes and allow a better distinction by IFM of PM bound and of cytoplasmic actin. When actin is detected by use of the sensitive fluoresceinated avidin-biotinylated myosin method, it then appears that only a fraction of the actin is redistributed as subcaps under the Con A caps and a substantial amount of the intracellular actin remains in the rest of the cell (54). Such association of subpatches of actin and myosin with patches of external PM components is obtained for a variety of different PM components in various metabolism-inhibited cells (16, 17, 54–56). It shows that external clustering of PM components by multivalent ligands leads to an energy-independent association with mechanochemical proteins prior to redistribution of the latter in the uropode, and it suggests that these Cs/Cm proteins actually induce movement of the patches into the cap area. Not all the actin is brought to the cap, and it would have been surprising indeed if all of a protein that represents 10–20% of total cell protein, and 10% of which is PM associated, were required for a process such as capping.

IV. Microvilli Formation and Shedding

Besides capping, microvilli (MV) formation and dynamics is one of the most remarkable activities of the cell cortex. The expression of these labile structures by lymphocytes is extremely variable. The causes of such variability are not all established; most of them seem to depend on environmental conditions (reviewed by 363, 531; see also 696), but others depend on the level of differentiation of the cell and the given phase of the cell cycle (148, 151, 322). *In vitro*, it is possible to modulate MV expression by various means. Expression of MV increases in the presence of a metabolic inhibitor such as 10 m*M* NaN_3 (127, 128, 368, 411) and generally when the level of ATP of the cell is decreased by use of inhibitors of respiration and glycolysis (127, 128). The presence of cytochalasin B in the medium can completely abolish the expression of MV on lymphocytes (368, 445) as well as on other cells (152, 444, 445). When cells expressing numerous long MV are treated with the drug, first their MV collapse, but later a number of