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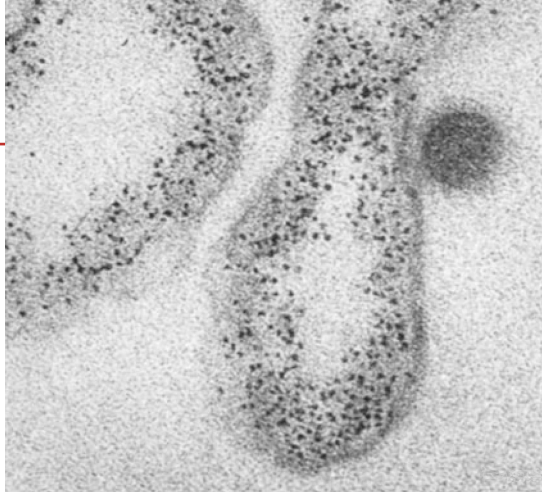
Spectrin is peripheral

In 1971, "next to nothing was known about the organization of membrane proteins," says S. Jonathan Singer (University of California, San Diego, CA). Singer had proposed that there were two kinds of membrane proteins—integral and peripheral—but the idea was, at the time, largely speculative (Singer, 1971). It was a collaborative study between Singer, his then graduate student Garth Nicolson, and Vincent Marchesi of the National Institutes of Health that provided strong evidence for the existence of peripheral proteins (Nicolson et al., 1971a).

Earlier work by Marchesi and Steers (1968) had shown that the protein spectrin was associated with the membranes of red blood cells. It could be isolated by mild treatments and behaved like a water-soluble protein. Some researchers thought spectrin was typical of membrane proteins in general. Singer's model, however, proposed that integral membrane proteins, which passed through the membrane, would be insoluble in water. In contrast, proteins like spectrin belonged to a distinct category. "I thought that spectrin would be peripheral to the membrane and attached to specific integral proteins where they stuck out from the membrane into the cytoplasm," recalls Singer.

To investigate this idea, Singer wanted to see where spectrin was located. He used his own ferritin-conjugated antibody technique (Singer, 1959) and got the antibodies into the red cell ghosts by fixing the ghosts while they had holes in their membranes from incubation in hypotonic medium (Seeman, 1967). Electron micrscopy (EM) analysis then localized the electron-dense ferritin-conjugated anti-spectrin antibodies specifically to the inner surface of the cell membrane (Nicolson et al., 1971a).



SINGER

Spectrin is localized to the inner membrane surface of red cell ghosts.

Marchesi and Palade (1967) had

A macrophage mystery leads to dendritic discovery

At the beginning of the 1970s, researchers thought that macrophages might help initiate the immune response, but the underlying mechanisms were obscure. Macrophages might process antigens, according to various theories, by producing immunogenic RNA (Askonas and Rhodes, 1965), acting as reservoirs that exocytosed antigen (Cruchaud and Unanue, 1971), altering antigens extracellularly (Shortman and Palmer, 1971), or, perhaps most popular, retaining whole protein antigen on their surface (Unanue et al., 1969).

Ralph Steinman remembers the puzzle surrounding the macrophage's role and how it might process antigens. He and Zanvil Cohn at the Rockefeller University in New York decided that the horseradish peroxidase (HRP) enzyme—newly adapted as part of an assay system for EM (Graham and Karnovsky, 1966)—would serve as both a model antigen and a clear and sensitive endocytic tracer. They reasoned that HRP's journey into the macrophage as an active enzyme would be easy to quantify and follow at the subcellular level. This represented one of the earliest forays of immunologists into the world of cell biology.

Although other studies had concluded that a small amount of intact antigen remained on the cell surface of macrophages (Unanue et al., 1969; Unanue and Cerottini, 1970; Schmidtke and Unanue, 1971), Steinman and Cohn could find no HRP bound extracellularly (Steinman and Cohn, 1972). Instead they saw HRP internalized, incorporated into secondary lysosomes, and presumably digested as activity fell off. In addition, radioactively labeled HRP appeared to be hydrolyzed down to single amino acids, which argued against macrophages retaining any antigenic information.

"At that time, the biological function of the major histocompatibility complex [MHC] had not been defined beyond its role in encoding antigens for transplant rejection," says Steinman. MHC function was defined in 1975 (Doherty and Zinkernagel, 1975; Zinkernagel and Doherty, 1975) and eventually led to the discovery of the "fascinating [antigen] processing pathway." Emile Unanue's group eventually showed that macrophage MHC could bind and retain antigen peptide fragments, which were recognized as peptide–MHC complexes by T cells (Allen et al., 1984).

In the days before MHC function is defined, digestion of antigens in macrophages is mistakenly taken as evidence against the cells' role in antigen presentation. But the study leads Ralph Steinman and Zanvil Cohn to their vital discovery of dendritic cells.

But in 1973 the concept of antigens being presented as peptide fragments "was not envisioned at all," says Steinman. Antigens were seen as a stable entity, so disappearance of HRP enzymatic activity was equated with disappearance of antigen. Steinman and Cohn used trypsin digestion to enhance release of any surface-bound HRP, but the tiny quantity of radioactive HRP peptide left on MHC would probably not have been detected by their methods—MHC is resistant to release by

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S. Jonathan Singer, Garth Nicolson, and Vincent Marchesi use red cell ghosts to provide strong evidence for the existence of peripheral membrane proteins.

first speculated that a kind of protein skeleton underneath the cell membrane provided mechanical strength. "We found very high concentrations of spectrin under the membrane. So we also thought it might be involved in what is now referred to as the membrane skeleton," says Singer.

Several years later these predictions were borne out. It is now known that spectrin is the most abundant peripheral membrane protein in red blood cells and the principal component of a protein meshwork, or membrane skeleton, that underlies the cell membrane. This membrane skeleton, which contains other proteins, including actin (Tilney and Detmers, 1975), restricts the lateral mobility of membrane-penetrating protein molecules (Nicolson et al., 1971b; Elgsaeter and Branton, 1973). It also maintains the structural integrity and biconcave shape of the red blood cell and explains why membranes are not mechanochemically identical to lipid bilayers (Evans, 1973). Peripheral proteins also exist beneath the plasma membrane of many nucleated cells, but these proteins form a discontinuous network under the membrane, do not include spectrin, and generally allow most integral proteins to diffuse globally in the membrane (Frye and Edidin, 1970). **LB**

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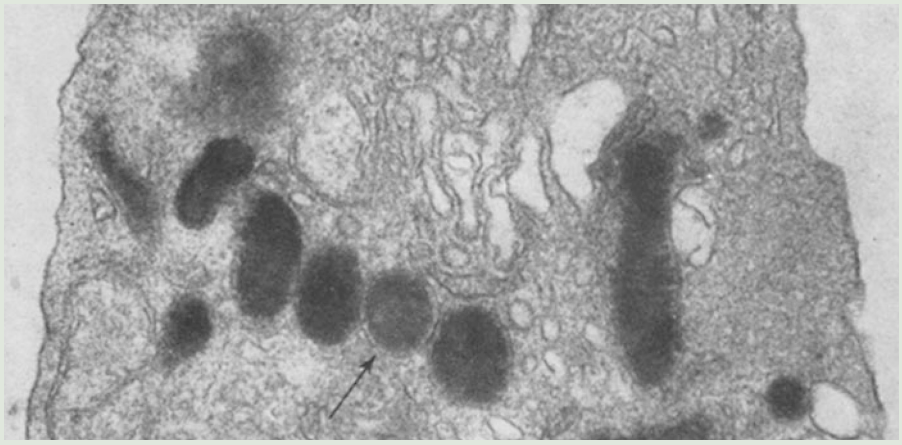

STEINMAN

Ingested HRP (black) appeared to be digested rather than processed.

the initiation of the immune response," says Steinman. "When we couldn't find retained antigen in macrophages, we thought maybe we were looking at the wrong cell and that took us to the spleen where we found dendritic cells." This related cell type turned out to be the "professional" antigen-presenting cell (Steinman and Cohn, 1973), and one of the key components of the immune system. It is that work—not the macrophage detour—that marks the highlight of Steinman's career. **KP**

proteases, and many antigenic peptides may have lacked the radioactive tags from the original proteins.

Steinman and Cohn's data had led them to have doubts about antigen processing by macrophages. Later it became clear that macrophages did present antigen as peptide–MHC, but meanwhile the work had several other, more positive consequences. The group used the HRP technique to track fluid and membrane movement, thus demonstrating that extensive membrane recycling must be happening at the plasma membrane. And the concept of presenting whole antigens was borne out in lymphoid areas rich in B cells, where intact, extracellular antigen is held on the surfaces of another cell type to stimulate B cells (Chen et al., 1978).

But for T cells, the action was elsewhere. "Our focus was

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