Review

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Initiation of Infection

The HIV-1-containing macrophage compartment: a perfect cellular niche?

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Macrophages are a major target of HIV-1 infection and are believed to act as viral reservoirs and mediators of HIV-1-associated neurological damage. These pathological roles may be associated with the ability of the virus to assemble and accumulate in apparently intracellular compartments in macrophages. These so-called virus-containing compartments were initially thought to be late endosomes or multivesicular bodies, but it has since been shown that they are distinct structures that have complex three-dimensional morphology, a unique set of protein markers, and features such as a near-neutral pH and frequent connections to the extracellular milieu. These features appear to protect HIV-1 from hostile elements both within and outside the cell. This review discusses the cellular and molecular characteristics of HIV-1-containing compartments in macrophages and how they offer a safe haven for the virus, with important consequences for pathogenesis.

Discovery of virus-containing compartments in macrophages

During HIV-1 infection of human hosts, the virus primarily targets two cell types: $CD4^+$ T cells and macrophages. A decline in $CD4^+$ T cell numbers is largely associated with the onset of immunodeficiency symptoms, whereas macrophages appear to act principally as viral reservoirs [1] and mediate virus-inflicted neurological damage [2], although substantially broader macrophage contributions to HIV-1 pathogenesis have been proposed [3–5]. Although it has been established that HIV-1 assembles at the plasma membrane of primary $CD4^+$ T cells, the corresponding site used in macrophages is still in the process of being characterized. Initial ultrastructural studies of HIV-1-infected macrophages revealed immature and mature virions in seemingly intracellular vesicles that resembled late endosomes (LEs) or multivesicular bodies (MVBs) [6,7]. Subsequent work using immuno-electron microscopy (immuno-EM) demonstrated that these compartments are enriched in MVB markers such as the tetraspanins CD9, CD53, CD63, CD81, and CD82, and MHC-II [8–10]. HIV-1 virions released from infected macrophages also possess these markers, apparently confirming that HIV-1 assembles in LEs/MVBs in macrophages [9,11]. However, the field was revolutionized when a series of studies published in 2007 demonstrated that these virus-containing compartments (VCCs) are not LEs or MVBs, because they have unique properties such as neutral pH and tubular connections to the extracellular surface [10,12,13]. Since then, these compartments have been further characterized and proteins important for their function as sites of HIV-1 assembly, budding, and storage have been identified. This review discusses these studies and the current state of knowledge of VCCs in macrophages. Because the mechanisms of HIV-1 replication and assembly vary greatly between different cell types and states of differentiation, we draw inferences principally from experiments conducted on primary monocyte-derived macrophages (MDMs), hereafter called macrophages unless otherwise stated, rather than model systems such as immortalized cell lines.

Properties of VCCs

VCCs are apparently intracellular compartments in macrophages that act as sites of HIV-1 assembly, because EM images depicting budding, immature, and mature virions at these locations have demonstrated that the compartments are not mere holding sites for the virus [9,10,13–15]. VCC-like compartments may not be formed specifically in response to HIV-1 infection because structures with similar morphology and marker composition exist in uninfected macrophages [10,13,15]. Their mechanism of formation and function pre-infection are unknown, but the compartments develop in macrophages as the cells differentiate [16] and become further enlarged on HIV-1 infection [15]. VCCs are not morphologically uniform: some resemble MVB-like compartments containing round or oval vesicles, whereas others are more sponge-like or consist of closely apposed membranes (Figure 1A–H) [10,12,17]. Three-dimensional studies of VCC topography have demonstrated that many of these seemingly distinct forms are actually different parts of the same three-dimensional structure(s), which is a complex tubulovesicular membranous web that extends throughout the macrophage (Figure 1I) [14,15,18]. VCCs are frequently connected to the extracellular milieu via narrow channels that are accessible to small membrane-impermeable dyes such as ruthenium red (RR) and horseradish peroxidase [10,13–15,18]. The level of antibody accessibility to VCCs is, however, unclear. Antibodies against tetraspanins were unable to enter VCCs during incubation for 90 min at 4°C [19] and neutralizing antibodies against gp120 could not penetrate the compartment when incubated for 1 h at

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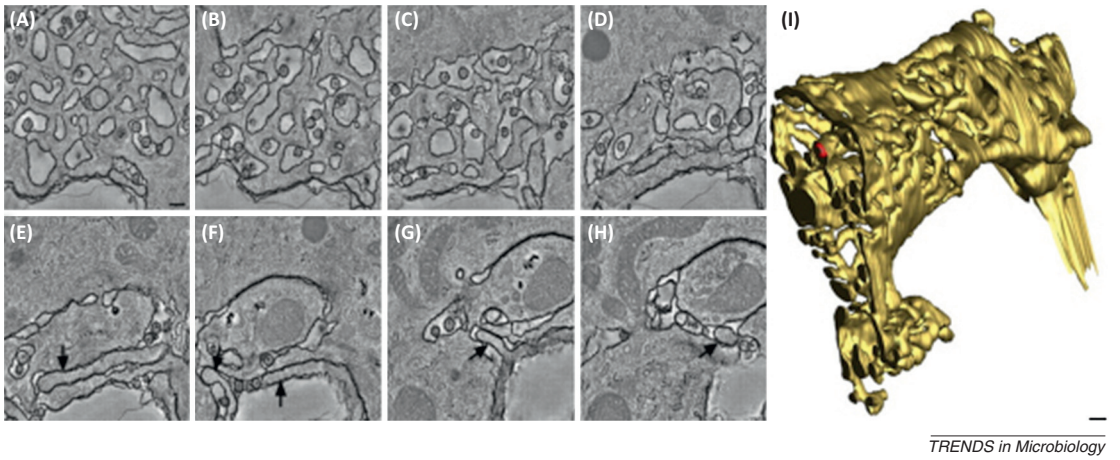

Figure 1. Virus-containing compartment (VCC) morphology. **(A–H)** Digital slices (from electron tomography) through a region of a HIV-1-infected macrophage depicting the morphological complexity and variability of the VCC. The arrows point to membranous protrusions that were initially thought to be a separate form of VCC but were later shown to be part of the overall three-dimensional VCC structure. **(I)** Three-dimensional reconstruction of a VCC containing a virus particle (red). Scale bars, 200 nm. Modified from [15] with permission.

37°C [18]. By contrast, tetraspanin-specific antibodies could be detected in VCC-like compartments in uninfected macrophages after incubation for 3 h at 37°C [10]. According to these findings, it may be that antibodies cannot enter VCCs at all, or antibody entry may be limited by the rate of diffusion through the narrow surface-connected channels. It is also conceivable that antibodies are taken up and trafficked to VCCs by an alternative rate-limiting mechanism such as FcR-mediated internalization. These possibilities should be evaluated in future experiments.

Available evidence suggests that not all VCCs are surface-accessible, because studies into VCC topology have consistently identified a coexisting population of seemingly enclosed VCCs [10,12,14,15,18]. It has not been established whether these two populations of VCCs represent separate entities or if they share a common origin. Nevertheless, it has been shown that they share several similar properties. First, surface-accessible and enclosed VCCs exhibit a range of sizes and types of morphology [13,15]. Second, neither surface-accessible nor enclosed VCCs belong to the endosomal system. Studies using internalized bovine serum albumin (BSA)–gold to label endosomes in macrophages revealed extremely low numbers of gold particles in both populations of VCCs but strong labeling in nearby endocytic compartments [12,13]. Third, VCCs have an almost neutral pH, in contrast to LEs and lysosomes, which are strongly acidic [12]. This has been attributed to the presence of surface connections [10,13] and/or a lack of recruitment of the acidifying enzyme V-ATPase to VCCs [12]. Finally, all VCCs share similar expression levels of the membrane marker CD44 with the plasma membrane, suggesting a cell surface origin, even for apparently enclosed VCCs [13]. By contrast, LEs have practically no CD44, further highlighting the difference between VCCs and endosomes [13].

The possibility of the two VCC populations being of different origins cannot be discounted, but if they are identical, several explanations could account for the apparent variation in surface connectivity. The VCC membrane may be highly dynamic, allowing intermittent opening and closing of the narrow channels connecting the compartments to the surface. If so, methods that take snapshots of the membrane such as EM would be unable to capture membrane movements and would only mark VCCs that are open at the time of fixing as surface-accessible. There are some indications that the VCC membrane may have dynamic flexibility. An early study investigating the entry of horseradish peroxidase into rabbit macrophages revealed rapid movement and fusion of surface-connected tubules with internal vesicles within minutes of incubation with the molecular marker [20]. In addition, the VCC membrane expands on HIV-1 infection, possibly by invagination of the plasma membrane and fusion with internal membranes [15]. The hypothesis of a highly dynamic VCC membrane would reconcile several discrepancies between different studies of VCC morphology, such as varying widths of surface-accessible channels (from 20 to 200 nm) [10,14,15] and whether only one or several separate surface-connected VCCs are present in a HIV-1-infected macrophage [14,15,18]. Another possible explanation for the differences in surface accessibility is directional maturation of a proportion of VCCs into closed compartments. This phenomenon has not been seen in HIV-1-infected macrophages, but closure of surface-connected compartments has previously been observed in macrophages fed with very-low-density lipoprotein [21]. Finally, RR, which is commonly used to delineate VCC membranes, may have limited diffusion activity, because the marker appears to stain peripheral VCCs more strongly than their more internal counterparts [13,15]. Thus, although absent RR labeling may well represent a dilution effect rather than the presence of closed vesicles, unequivocal interpretation of these data awaits careful quantification of RR labeling at plasma membrane-proximal and -distal membrane surfaces.

HIV-1 assembly and release

Although it is well established that HIV-1 virions can assemble and bud at VCCs in infected macrophages, a more contested question is whether viral assembly can also take place at the plasma membrane. Several groups