Published in final edited form as:

Immunol Res. 2009 ; 43(1-3): 210–222. doi:10.1007/s12026-008-8072-7.

Endocytosis as a mechanism of regulating natural killer cell function: unique endocytic and trafficking pathway for CD94/NKG2A

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Abstract

Natural killer (NK) cells are lymphocytes generally recognized as sentinels of the innate immune system due to their inherent capacity to deal with diseased (stressed) cells, including malignant and infected. This ability to recognize many potentially pathogenic situations is due to the expression of a diverse panel of activation receptors. Because NK cell activation triggers an aggressive inflammatory response, it is important to have a means of throttling this response. Hence, NK cells also express a panel of inhibitory receptors that recognize ligands expressed by “normal” cells. Little or nothing is known about the endocytosis and trafficking of NK cell receptors, which are of great relevance to understanding how NK cells maintain the appropriate balance of activating and inhibitory receptors on their cell surface. In this review, we focus on the ITIM-containing inhibitory receptor CD94/NKG2A showing that it is endocytosed by a previously undescribed macropinocytic-like process that may be related to the maintenance of its surface expression.

Keywords

NK cells; Inhibitory/activating receptors; Endocytosis; Trafficking; CD94/NKG2A

Introduction

Natural killer (NK) cells are large granular lymphocytes with the inherent ability to kill target cells (e.g., tumor or virally-infected cells) and/or secrete cytokines [1]. Generally recognized as sentinels within the innate immune system, they express a large variety of activating receptors on their surface capable of recognizing target cells [2]. Due to the fact that the majority of normal cells express some of the ligands for these receptors, a mechanism to avoid

autoimmune responses must exist. Consequently, NK cells also express inhibitory receptors whose signals are able to override basal activation signals [3,4]. The ligands for the predominant inhibitory receptors are major histocompatibility complex (MHC) class I molecules that are expressed by most normal cells. Potential target cells must express sufficient levels of ligands for activating receptors relative to inhibitory ligand expression to override this inhibition in order to stimulate NK cell activation. In this light, any down-modulation of inhibitory receptor expression by NK cells would lower the threshold for NK cell activation possibly making normal cells vulnerable to attack.

In general, upon interaction with their specific ligands, receptors undergo down-modulation and are routed to lysosomes for degradation, a mechanism recognized as useful to dampen the intensity and duration of signaling to regulate activation responses [5]. This creates a conundrum of explaining how NK cells maintain surface expression of inhibitory receptors in a milieu of surrounding cells that under normal circumstances express inhibitory receptor ligands. Little is known regarding the endocytosis and trafficking of NK cell surface receptors. Here, we present a general overview on receptor trafficking routes emphasizing our description of a novel endocytic/trafficking pattern for the CD94/NKG2A inhibitory receptor [6].

NK cell receptors ligation and activation

Both inhibitory and activating NK cell receptors initiate intracellular signaling through specific amino acid sequence motifs contained in their cytoplasmic tails or their associated adapter proteins, respectively. Like many B and T cell antigen receptors, most NK cell activating receptors transduce an intracellular signal through assorted adapter proteins containing one or more immunoreceptor tyrosine-based activation motifs (ITAM) defined by the sequence Asp/Glu-x-x-Tyr-x-x-Leu/Ile, with x representing most other amino acids [7]. Depending on the receptor, these transmembrane-anchored adapter proteins can be DAP12, Fc ϵ RI- γ , or CD3- ζ . Receptor ligation leads to the phosphorylation of the Tyr residue within the ITAM motif, probably by Src family kinases. The subsequent recruitment of tyrosine kinases, such as Syk and/or Zap-70, via their SH2 domains leads to the propagation of a complex pattern of downstream signaling events that promote actin cytoskeleton reorganization, and induction of degranulation and/or the transcription of cytokine and chemokine genes [4,8]. Unlike most activating receptors, such as CD16, CD94/NKG2C, KIR, NKp30, NKp44, NKp46 [4], NKG2D initiates signaling through its association with DAP10 that contains a YxxM-motif; phosphorylation of the Tyr residue activates the Syk-independent, PI3K/Grb-2 signaling pathway [9].

Recent research indicates that most NK cell effector functions are not mediated by a single activating receptor, but most probably from the ligation of a combination of receptors [10]. However, the low affinity Fc receptor for IgG CD16 (Fc γ RIII) [11], responsible for antibody-dependent cellular mediated cytotoxicity (ADCC) is apparently able to elicit NK effectors function without the simultaneous cross-linking of additional receptors.

The intracellular signaling of inhibitory receptors is mediated by immunoreceptor tyrosine-based inhibitory motifs (ITIM) that are characterized by the sequence Iso/Leu/Val/Ser-x-Tyr-x-x-Leu/Val with x representing most other amino acids [12]. Upon the interaction of inhibitory receptors with their ligands, ITIMs are tyrosine phosphorylated and act as docking sites for SHP-1, SHP-2, and/or SHIP-1 phosphatases. These tyrosine phosphatases are able to terminate NK cell effector function by dephosphorylating the protein substrates of the tyrosine kinases linked to activating NK receptors [13,14]. A recent publication suggests that the binding of the phosphatase to the phosphorylated ITIM may be mediated by β -arrestin 2 [15]. In addition, a recent paper suggests that phosphorylated Ser residues outside the ITIM motif of KIR3DL1 may play a role in inhibitory receptor function [16].

The fact that NK inhibitory receptors recognize major histocompatibility complex (MHC) class I-type molecules that tend to be expressed by all normal cells suggests that this recognition process is important for controlling the potentially harmful lytic and inflammatory tendencies that NK cells possess toward all cells that they encounter [17]. In humans, the predominant NK inhibitory receptors interacting with MHC class I molecules are the heterodimeric CD94/NKG2A (and the alternative spliced form NKG2B), the killer cell Ig-like receptors (KIR) and the leukocyte immunoglobulin-like receptors (LILRs or ILTs). While the ligands for the type I transmembrane glycoproteins KIR and LILRs are mainly the classical human leukocyte antigen (HLA) class I molecules (HLA-A, B, and C), the ligand for CD94/NKG2A is the non-classical class I molecule HLA-E [18].

Routes for endocytosis and trafficking

As pointed out, NK cells must be finely regulated so that they aggressively respond to “abnormal” cells, but not to the extent that the resultant response becomes self-destructive. Maintenance of inhibitory receptor surface expression is critical for self-protection. In this light, it is important to understand the endocytic/trafficking process that functions to maintain constant inhibitory receptor expression.

Receptor endocytosis can be constitutive or ligand-induced [19]. Constitutive endocytosis occurs at a defined rate regardless of bound ligand, whereas ligand-induced endocytosis occurs upon receptor interaction with specific ligands. Receptors undergoing constitutive endocytosis are generally non-signaling ones that mediate the uptake of nutrients. The best example of this is the transferrin receptor (Tf-R). On the other hand as mentioned, receptors that “activate” cells tend to be down-regulated by endocytosis to prevent excessive responses, as exemplified by growth factor receptors like EGFR.

Two general types of endocytosis are recognized: phagocytosis that occurs in professional phagocytes and pinocytosis that occurs in most mammalian cell types. Currently the pinocytic pathway can be divided into the clathrin-dependent pathway and three clathrin-independent internalization pathways, namely, macro/micropinocytosis, caveolae-dependent internalization, and a pathway independent from both termed the GPI-enriched endosomal compartments (GEEC) pathway [20]. Internalization is just the beginning of the complex endocytic journey of receptors within the cell. In Fig. 1, we illustrate the potential endocytic routes and intracellular compartments available for receptor trafficking.

Distinguishing endocytic routes

As mentioned, receptors can be endocytosed into the cell by a variety of pinocytic mechanisms [21,22]. Of these, clathrin-mediated endocytosis is perhaps best understood. This process is thought to be initiated by adaptor proteins that localize to the plasma membrane through phosphatidylinositol-4, 5-bisphosphate-binding sites and that are also capable of recognizing sequence-specific motifs in the cytoplasmic tails of receptor proteins. The receptor-associated adaptor complex then recruits clathrin to the plasma membrane and promotes its assembly into a polygonal lattice leading to the formation of clathrin-coated transport vesicles [23,24]. Although AP-2 is the major adaptor protein found in clathrin-coated pits, a number of accessory proteins can act together with AP-2 or act as alternative adaptors. Epsin, β -arrestin, AP180/CALM, Dab2, and Hip1 have been shown to interact not only with AP-2 but also with phosphatidylinositol-4, 5-bisphosphate and clathrin, which indicates that they may be able to bind to the plasma membrane and recruit clathrin independently of AP-2 [23,24]. There is also evidence that each of these proteins can recognize specific internalization signals on the receptor cytoplasmic tail that differ from those recognized by AP-2 [23].

Transferrin, through association with the Tf-R receptor, is specifically internalized via a clathrin-dependent mechanism and thus can be utilized as a marker for clathrin-dependent intracellular pathways. Hypertonic treatment of cells with 0.45 M sucrose is used to inhibit clathrin-mediated endocytosis [25,26], but a more specific means is through treatment with specific siRNAs to deplete clathrin heavy chain (CHC) [6,27] or the α -adaptin and μ 2-subunit of the AP-2 adaptor complex [27]. The GTPase dynamin is targeted to coated pits through interactions with amphiphysin, which also binds AP-2 and clathrin [28,29]. Dynamin facilitates the endocytic process by promoting membrane invagination and fission of cargo bearing clathrin-coated vesicles from the plasma membrane. Because of the process(es) it regulates, dynamin has been shown to be involved in a broad spectrum of endocytic events including caveolae/raft-mediated internalization, phagocytosis, and fluid-phase pinocytosis, as well as actin cytoskeleton reorganization [29-32].

Multiple cell surface receptors, such as GPI-anchored proteins, MHC class I molecules [33, 34], several types of G-protein-coupled receptors [35], and the γ -chain of IL-2 receptor [31] seem to require intact lipid rafts to be endocytosed [36]. It has been generally observed that rafts and raft-associated proteins are endocytosed by non-clathrin-mediated pathways [36-38], but exceptions to this have been reported [39].

Although many studies have demonstrated the existence of lipid rafts, a lot of controversy exists regarding their contents and distribution. Keeping this in mind, lipid rafts are generally characterized as domains present in the membrane usually rich in saturated lipids, cholesterol, and proteins involved in the signal transduction, as for example G proteins or glycosylphosphatidylinositol (GPI)-anchored proteins [40]. Different methods and markers are utilized to characterize them. One method used for their isolation consists of extracting detergent-resistant membrane (DRM) fraction with Triton X-100 [41]. The presence of receptors in this fraction indicates that they are raft-associated [6,27]. Markers generally used to identify lipid domains are GM1 ganglioside [42] or Xotillin-1 [43]. Flotillin-1 appears to identify an endocytic pathway distinct from clathrin and caveolae endocytosis [43]; it is important to be aware that flotillin proteins can be found in most endocytic compartments and even in the nucleus [44].

Caveolae are special type of lipid rafts, whose main components are caveolin proteins 1, 2, and 3 that are abundant in specific cell types, such as fibroblasts, endothelial cells, and adipocytes but absent in lymphocytes. Caveolin-1 plays a pivotal role in the caveolae vesicle formation as demonstrated by the lack of caveolae in caveolin-1-knock out mice [45]. Dynamin is involved in the internalization step of caveolae [46]. And recent data have shown that caveolin-positive vesicles can interact with early endosomes in a Rab5-dependent process [47]. Cholera toxin and transforming growth factor β (TGF- β) receptor are examples of cargoes that are internalized via a caveolae-dependent process. Actually, TGF- β can be internalized by both the clathrin-dependent and caveolae-dependent pathways [48].

Most of the endocytic pathways described above are dynamin-dependent, but there are a growing number of descriptions of dynamin-independent endocytic routes for various cell surface proteins. These pathways are also clathrin and caveolin independent and are regulated by a variety of small GTPases [49]. For example, the Arf6 GTPase regulates the internalization of MHC Class I molecules [34]. Others have shown that the Rho family GTPase Cdc42 is responsible for the uptake of specific membrane components, such as GPI-anchored proteins and cholera toxin bound to GM1 [49,50], by a distinct pathway that does not require Arf6 function. Pinocytic vesicles devoid of clathrin and caveolin, termed clathrin- and dynamin-independent carriers (CLICs) [51], mediate the uptake and then fuse to form endosomal compartments termed GPI-anchored protein enriched early endosomal compartments (GEECs)

[49,52]. This pathway is also sensitive to cholesterol depletion and is inhibited by incubation with inhibitors of actin polymerization such as latrunculin A and cytochalasin D [49].

It is becoming clear that receptors are not tied to a single endocytic mechanism and that there may be cooperation among the various internalization pathways. A good example of this is the B cell receptor (BCR) which upon binding to antigen was observed to be internalized via lipid rafts or clathrin-coated pits by Putnam et al. [53]. A more recent study [54] showed that the most extensive BCR internalization occurs when clathrin cooperates with lipid rafts and the actin cytoskeleton. Ligated FcεRI is another example of a receptor that can apparently be internalized by either a clathrin-mediated [55] or lipid raft-mediated [27] mechanism.

Macro- and micropinosome formation resembles the ruffling involved in lamellipodia formation [56]. The engulfment of extracellular fluid-phase markers, such as fluorophore-conjugated dextrans and lucifer yellow, are usually used as markers to identify fluid-phase pinocytic processes. Macro- and micropinosome are morphologically distinguished by their size, greater or lesser than 0.2 μm [56]. It is not yet clear how many types of pinocytic vesicles exist and how many pathways are involved in their endocytosis [57]; however, it is clear that distinguishing these pathways by the size of the endocytic vesicle utilized is somewhat arbitrary [56].

The formation of macropinocytic vesicles have been shown to involve actin polymerization [56,58] and small GTPases, such as ADP-ribosylation factor 6 (Arf6) [59,60] and Rac1 [61]. Moreover, the process is regulated by PI3K [58,62] and, consequently, macropinocytosis can be transiently stimulated by phorbol esters, like PMA, that activate the protein kinase C [56]. The use of actin-disrupting drugs, such as latrunculin A and cytochalasin D or aluminum Xuoride (AlF₄⁻), that perturbs Arf6 function [60] or the PI3 kinase inhibitor wortmannin [62] are tools generally used to study a macropinocytic process. Also, the macropinocytic mechanism is sensitive to inhibitors of Na⁺/H⁺ exchange, such as amiloride [63-65]. Thus, beside the size of the pinocytic vesicles, micropinocytosis is distinguished from macropinocytosis by its PI3K and actin independency [66], and resistance to AlF₄⁻ and amiloride exposure.

Ubiquitination is a post-translational and reversible modification in which a single (mono-) or a chain (poly-) of ubiquitin molecules (Ub) is added to a substrate protein. Such modifications can not only regulate protein degradation and signaling processes, but also protein endocytosis [67-69]. For example the epidermal growth factor receptor (EGFR) undergoes a clathrin or caveolae-dependent endocytosis based on the levels of extracellular ligand and ubiquitination [70,71]. At high doses of EGF, receptors undergo monoubiquitination mediated by the E3 ubiquitin ligase Cbl and are mainly internalized via caveolae. At low (physiological) ligand doses, EGFR internalizes through a clathrin-dependent pathway and it is not ubiquitinated.

In the end, the characterization of endocytic machinery is an evolving process [72] that will probably lead to continued refinement of our understanding of the mechanisms involved.

Intracellular trafficking

Generally, after endocytosis, receptors are first delivered to early endosomes localized toward the cell periphery and characterized by mildly acidic pH that generally promotes receptor dissociation from its ligand. From the early endosomes, also named the sorting endosomes, the internalized receptors are either recycled to the plasma membrane or delivered to the lysosomes for degradation [5]. In the former case, the receptors are either directly recycled to the plasma membrane or sorted through the recycling endosome. In the latter case, the receptors are translocated to the more acidic and spherical compartments, the so-called endosomal carrier vesicles or multivesicular bodies (ECV/MVBs). These endosomes that share a diameter of

400–500 nm translocate the majority of the receptors along microtubules to the late endosomes with which the ECV/MVB fuse. Late endosomes share the presence of cisternal, tubular, and multivesicular regions and their shape is highly pleiomorphic. Receptors destined for degradation are then transferred from late endosomes into the lysosomes located closer to the nucleus; however, some of them can still recycle back to the cell surface from these locations [73].

The traffic of internalized cargo proteins within the various endosomal compartments can be followed using specific markers. A well-established marker for endocytic compartments is the early endosomal antigen 1 (EEA1) that marks early endosomes. Several small GTPases of the Rab family regulate transport among these organelles and specifically mark different endocytic compartments [74]. Rab4 and Rab11 are specific markers of the recycling endosome and modulate the recycling from this compartment to the plasma membrane. At times, Rab4 can also be found within early endosomes presumably related to the delivery of the receptors into the recycling compartment [75]. Rab5 is a specific marker of early endosomes and Rab7 is a specific marker of late endosomes. LysoTracker and lysosome-associated membrane protein (Lamp)-1 and -2 identify lysosomes and late endosomes, and CD63 identifies all the late endosomes including multivesicular bodies. Since the trafficking pattern of the Tf-R has been well characterized, whether or not a receptor co-localizes with internalized Tf-R can provide valuable information about the trafficking of that receptor. Tf upon association with Tf-R, depending on the cell type and the time of treatment, 10 min or 1 h, is generally used to mark the early endosomes or the recycling endosome, respectively [76].

Receptor trafficking can vary depending on various factors, such as post-translational modifications or the nature of bound ligands. A common post-translational modification of trafficking proteins is ubiquitination that generally targets proteins for degradation by proteasome or by lysosomes [67,77]. The endocytic process can also be regulated by differential binding of the receptors to its multiple ligands. As an example of ligand-mediated trafficking, it has been shown that the keratinocyte growth factor receptor/fibroblast growth factor receptor 2b (KGFR/FGFR2b) can be activated by two different ligands, the keratinocyte growth factor (KGF)/fibroblast growth factor (FGF) 7 and the FGF10/KGF2. Both ligands induce KGFR/FGFR2b clathrin-dependent internalization. In the case of KGF/FGF7, the receptor is ubiquitinated by c-Cbl and degraded at level of the lysosomes, as demonstrated by the co-localization of KGFR with both CD63- and lysotracker-positive vesicles. KGFR ligated with FGF10/KGF2 is not ubiquitinated and is delivered to the recycling endosome, as shown by the co-localization with Tf and absence of co-localization with either CD63 or lysotracker [76].

A unique trafficking route maintains CD94/NKG2A surface expression

CD94/NKG2A is an inhibitory receptor and in humans its ligand is HLA-E [18,78]. It is expressed by NK cells, certain populations of CD8 T cells, and, at times, CD4 T cells. HLA-E is expressed by most normal cells thereby protecting themselves from NK cell aggression by its interaction with CD94/NKG2A. HLA-E, along with other MHC Class I molecules, tends to be down-regulated by virally infected and tumor cells, presumably as a mechanism to avoid recognition by cytotoxic T cells (CTL); however, such cells may no longer have sufficient inhibitory ligands to protect them from NK cell attack [79,80].

Ligation of CD94/NKG2A by HLA-E leads to the phosphorylation of Tyr within the ITIM motifs present in the NKG2A intracellular tail that now can bind SHP-1 and/or SHP-2 [13, 80]. Activated phosphatases lead to the dephosphorylation of the Vav and ezrin/radixin/moesin (ERM) proteins that leads to the disruption of the actin cytoskeleton. This disrupts the signaling potential of activation receptor-bearing lipid rafts at the site of NK cell/target cell contact

[79,81,82]. Because of its vital role in suppressing the tendency of NK cells to attack normal cells, CD94/NKG2A cell surface expression needs to be maintained while being constantly exposed to ligand expressed by surrounding cells. In agreement with this hypothesis we showed that CD94/NKG2A is not down-regulated upon interaction with the ligand, but it is long lived and continuously recycles back to the cell surface, and traffics intracellularly through different compartments than the Tf-R and the CD94/NKG2C activating receptor [83]. This is in marked contrast to the NK cell NKG2D activating receptor, like CD94/NKG2A a member of the NKG2 C-type lectin family, which is down-regulated upon ligation [84-87] apparently by a clathrin-mediated process [87] that seems to be actin dependent [6].

As previously pointed out, to avoid the auto-reactive tendency of NK cells, it is important to understand the endocytic and trafficking processes that cooperate to maintain constant inhibitory receptor expression. Thus, we decided to study the trafficking process that supports the resilient surface expression of CD94/NKG2A [6]. Our data indicate that the endocytosis of CD94/NKG2A is clearly a pinocytic process as it is co-endocytosed with fluid-phase markers. The fact that CD94/NKG2A endocytic vesicles seems to be much greater in diameter (0.5–1.5 μm) than micropinosomes ($<0.2 \mu\text{m}$) characterizes them as macropinosomes [72]. The fact that internalized CD94/NKG2A co-localizes with dextran and lucifer yellow, and that its endocytosis is amiloride sensitive and Rac1 dependent supported this conclusion [63,88]. In human umbilical vein endothelial cells, the clustered cell adhesion molecules ICAM-1 and platelet endothelial cell adhesion molecule-1 are internalized by a macropinocytic mechanism that is independent of clathrin, caveolin, and PI3K activity; however, this internalization process requires actin and dynamin activity [63]. The use of RNA interference to suppress the expression of clathrin heavy chain (ch1 and ch2) and hypertonic treatment of cells did not affect CD94/NKG2A endocytosis demonstrating that the receptor is not internalized with a clathrin-dependent mechanism [6]. Moreover, Rac-1 activity is required for CD94/NKG2A internalization. Evidence that CD94/NKG2A internalization does not require actin, dynamin, or Arf6 and is insensitive to PI3K inhibition and PKC stimulation clearly distinguishes it from previously described macropinocytic mechanisms. On the other hand, despite the fact that CD94/NKG2A-containing endocytic vesicles are too large to fit the definition of a micropinosomes, they do share, in addition to PI3K independence, the notable feature of actin independency with the micropinocytic process. Keeping this in mind, considering the “plasticity” of endocytic process that can take place, we arbitrarily chose to term CD94/NKG2A endocytosis as *macropinocytic-like* realizing that it is clearly biochemically distinguishable from previously described macropinocytic mechanisms. This novel endocytic process is coupled to an abbreviated intracellular trafficking pattern in which endocytosed CD94/NKG2A enters the early endosomal compartment, but does not enter late endosomes nor does it fully enter the recycling compartment. Co-localization was in fact observed with EEA1 and Rab5, only partially with Rab4 and never with Rab7, Rab11, or Lamp-1 and 2. Figure 2 shows a schematic model of the CD94/NKG2A endocytic route. In the end, the *macropinocytic-like* pathway seems to be a process utilized by NK cells for its function and homeostasis. If this pathway turns out to be unique to CD94/NKG2A, it would indicate how crucial this protein is for NK cell function. Moreover, if the *macropinocytic-like* pathway seems to be utilized by others inhibitory receptors in general, it would emphasize the importance, efficiency, and plasticity of cells to adopt such energy saving tactics for its functional homeostasis.

Our current aim is to investigate whether other inhibitory ITIM-bearing receptors expressed on NK cells that function similarly to CD94/NKG2A are also endocytosed via an actin-independent process and whether they traffic similarly to CD94/NKG2A. Our work is currently focused on the study of the intracellular trafficking of the inhibitory leukocyte-associated Ig-like receptor-1 (LAIR-1) receptor, expressed by most lymphocytes [89].

Conclusions and future directions

Knowledge endocytosis and trafficking of activating and inhibitory receptors on NK cells and other lymphocytes is important for understanding how regulation of receptor expression within the endocytic compartments relates to the functional status of these cells. Our recent identification of a novel endocytic route and trafficking pattern for CD94/NKG2A leads us to hypothesize that inhibitory receptors may have a unique trafficking pathway. We plan to test this hypothesis by studying the bioprocessing of other ITIM-bearing inhibitory receptors expressed by lymphocytes, such as LAIR-1 (CD305) and CD300a. We also plan to look at ITIM-bearing inhibitory receptors on other cell-types, e.g., CD300lf on myeloid cells. Continued research on the trafficking pathways of NK cell receptors may enable us to experimentally alter these pathways in disease conditions that could result in better treatment strategies for cancer and autoimmune diseases.

Acknowledgment

This work was supported by the intramural program of the NIAID/NIH.

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Fig. 1.

Schematic representation of the main endocytic pathways. The figure shows the main endocytic pathways and intracellular routes that a receptor can undergo. In the first step of endocytosis a receptor can undergo clathrin-dependent (clathrin-coated pit formation) or clathrin-independent internalization (micro- or macropinocytosis and caveolae-dependent internalization) or a pathway independent from both (GEEC). Following internalization, receptors carried by different vesicles can enter the early (or sorting) endosomes from where they are segregated into separate trafficking itineraries. A receptor can recycle back to the plasma membrane directly from this compartment or after being sorted into the recycling endosome or continue to traffic to the lysosome for the final degradation passing through the endosomal carrier vesicles/multivesicular bodies (ECV/MVBs) and late endosomes. Recycling can also occur at level of late endosomes and lysosomes. Specific markers for each compartment are indicated in red. Recycling pathways are indicated with red lines (The color version can be viewed online in the electronic version of the manuscript.)

Fig. 2.

CD94/NKG2A intracellular trafficking. The endocytic route of CD94/NKG2A is schematically represented. We have no direct evidence of receptor association with membrane ruffles. The CD94/NKG2A positive vesicles that are formed near the plasma membrane are morphologically similar to macropinosomes in size. However the CD94/NKG2A internalization process does not require actin, dynamin, or Arf6; it is insensitive to PI3K inhibition and PKC stimulation and requires the involvement of Rac1. Thus we arbitrarily termed it a macropinosytic-like process. Upon 15–20 min of endocytosis, the receptor colocalizes with EEA1 and Rab5 and only minimally with Rab4. Co-localization with Rab7 or Rab11 and Lamp-1 or Lamp-2 is never observed indicating that the receptor is recycled back to the plasma membrane without entering the Rab7⁺ or Rab11⁺ compartments (red line) (The color version can be viewed online in the electronic version of the manuscript.)