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Single Particle Tracking reveals two distinct environments for CD4 receptors at the surface of living T lymphocytes

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ABSTRACT

We investigated the lateral diffusion of the HIV receptor CD4 at the surface of T lymphocytes at 20 °C and 37 °C by Single Particle Tracking using Quantum Dots. We found that the receptors presented two major distinct behaviors that were not equally affected by temperature changes. About half of the receptors showed a random diffusion with a diffusion coefficient increasing upon raising the temperature. The other half of the receptors was permanently or transiently confined with unchanged dynamics on raising the temperature. These observations suggest that two distinct subpopulations of CD4 receptors with different environments are present at the surface of living T lymphocytes.

1. Introduction

The process of HIV infection requires the sequential interaction of multiple receptors at the plasma membrane. Firstly, the gp120 viral envelope protein interacts with a CD4 receptor present at the surface of the target cell. Secondly, the same gp120 viral protein interacts with co-receptors (either CCR5 or CXCR4 for R5- and X4-virus, respectively) also expressed on the target cell. These successive interactions lead to protein conformational changes and, eventually, to the initiation of the fusion of viral and cellular membranes by the viral gp41 protein. This whole process finally leads to the release of the viral content into the cytoplasm of the cell (for review, see [1]).

It is now well known that the plasma membrane displays a dynamical organization with domains scaling from the nanometer to the micrometer range [2] and that this compartmentalization is essential for the regulation of many cellular functions [3,4]. Several authors have proposed that the existence of a compartmentalization of the HIV receptor and co-receptors in the plasma membrane of target cells might facilitate HIV infection [5–8]. Indeed, the virus may take advantage of such a clustering to quickly establish the numerous interactions required for membrane fusion and the release of its content into the host cell. Indications supporting this hypothesis have been reported by different groups. For example, it has been shown that the efficiency of HIV infection was dependent on receptor surface density [9]. Additionally, Singer and collaborators revealed by electron microscopy that CCR5, CD4 and CXCR4 form micro-clusters at the surface of primary macrophages and T cells [10] and our group demonstrated that a constitutive interaction of CD4 and CCR5 occurs in micrometer sized domains at the surface of stably transfected HEK cells [5,11].

A thorough analysis of the dynamical behavior of the HIV receptors within the membrane is needed for a better understanding of the early steps of the infection process. So far, however, only a few studies have focused on the dynamics of HIV receptors [5,12] and data on the dynamical membrane organization of receptors at the surface of natural targets of HIV (i.e. lymphocytes or macrophages) are still incomplete. In this study, we have used Single Particle Tracking (SPT) to study the dynamical organization of the CD4 receptor on $CD4^+$ T lymphocytes with a high spatial and temporal resolution.

2. Material and methods

2.1. Cell culture

The J.CCR5 cell line (a generous gift from F. Bachelerie) is a Jurkat cell line stably expressing CCR5 after transduction by a lentiviral vector. J.CCR5 cells were cultured at 37 °C and in a 5% CO_2 atmosphere in RPMI 1640 without L-glutamine (Gibco, Invitrogen) supplemented with 10% fetal calf serum (Lonza), and every 2 weeks with 2 mM L-glutamine (Gibco, Invitrogen).

2.2. Flow cytometry analysis

Cell surface expression of the receptors was determined as described previously [11] using a BD Biosciences FACS-Calibur.

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Labeling of the receptors was performed using biotinylated OKT4 anti-CD4 and T21/8 anti-CCR5 antibodies (BioLegend) coupled to streptavidin conjugated to phycoerythrin (BD).

2.3. Single Particle Tracking experiments

Cells were plated on coverslips previously incubated with 0.1 mg/ml poly-L-lysine for 5 min. For a homogenous attachment of the cells, a gentle centrifugation (50*g*, 7 min) was performed.

The CD4 receptors were labeled for 15 min with 0.03 nM biotinylated OKT4 antibodies (BioLegend) coupled to 0.3 nM fluorescent (655 nm) streptavidin-coated Quantum Dots (QD) (Molecular Probes, Life Technologies).

Tracking and observations were performed on an Axioplan 2 microscope (Zeiss) equipped with a Cascade II 512 EM-CCD camera (Roper Scientific) operating at a 25 Hz acquisition frequency. The fluorescent nanoparticles were illuminated with an X CITE 120 light source containing a metal halide vapor short arc lamp (Exfo), and observed through a fluar 100×/1.30 oil UV objective associated to a 1.6× multiplier tube lens in front of the camera.

2.4. Trajectories analysis

In a first step, the positions of the multiple QD present in each image have been calculated by a Gaussian fit using the Multiple Target Tracing program developed by Sergé et al. [13]. After obtaining the trajectory of each particle, the Mean Square Displacement (MSD) (Fig. 2B right) was calculated according to Eq. (1) [14,15]:

$$\mathrm{MSD}(n\delta t) = \frac{1}{N-1-n}\sum_{j=0}^{N-1-n}\Big\{[x(j\delta t + n\delta t) - x(j\delta t)]^2 + \Big[y(j\delta t + n\delta t) - y(j\delta t)\Big]^2\Big\} \quad (1)$$

where δt is the time interval between two successive frames (40 ms), n is the number of time intervals, $x(t)$ and $y(t)$ are the QD coordinates at time t and N is the total number of frames.

The short term diffusion coefficient of a given trajectory segment, $D_{1\text{-}2}$, was determined from the slope of the first two points of the corresponding MSD [16].

To detect transient or continuous confinement events, a confinement index $\Lambda(t)$, as established by Meilhac et al. [17] was calculated on sliding intervals using Eq. (2) (Fig. 2B, center):

$$\Lambda(t) = \frac{D_{1\text{-}2}\Delta t}{\Delta r^2} \quad (2)$$

where Δr^2 is the variance of the trajectory segment of duration Δt under study. A value of $\Lambda(t) > 4$ for a period longer than Δt is characteristic of confined diffusion. The size of the domains and the diffusion coefficient inside the confined trajectory segments were determined by fitting MSD(t) with its theoretical expression for confined diffusion [17]. The unconfined trajectories, partial or total, were analyzed using standard procedures [18] and classified as having either random or directed diffusion.

This analysis allowed classifying CD4 dynamics into different categories, namely random, confined, transiently confined or directed diffusion (see [19] for review).

3. Results and discussion

3.1. Choice and characterization of the lymphocyte cell line

It has been shown that HIV replicates most efficiently in activated $CD4^+$ T cells [20–22]. The activation process of these cells is accompanied by the expression of surface molecules such as HLA-DR and CD25 but also the HIV co-receptor CCR5 [23]. Additionally, it has been shown that the presence of CCR5 at the cell surface is required for the fusion of viral and cellular membranes and thus for the entry of the R5-virus into the cells [24–26]. As a consequence, HIV infection predominantly involves activated lymphocytes (i.e. that express CCR5) like those present at the gastrointestinal mucosa [21,27].

Unfortunately, upon activation of T cells, CD4 and CCR5 expression rates vary drastically, not only over time but also from cell to cell, making the interpretation of experiments very difficult. To overcome this problem, we have chosen to work on a Jurkat cell line (T lymphocytes) stably transfected by a lentiviral vector containing the CCR5 gene (referred to as "J.CCR5" cell line). As shown in Fig. 1, this cell line expresses both CD4 and CCR5 and was shown to be competent for HIV infection (data not shown). Owing to the stable expression levels of both CD4 and CCR5 over time and from cell to cell, this cell line was suitable to perform SPT experiments.

3.2. Imaging and tracking of CD4 in living cells

To investigate the movements of CD4 at the surface of J.CCR5 living cells, we observed, as a function of time, CD4 receptors labeled by a streptavidin-coated quantum dot (QD) coupled to a biotinylated-OKT4 antibody. The OKT4 antibody has been chosen since it does not inhibit CD4-CCR5 nor CD4-gp120 interactions [28,29]. QD are very stable fluorescent nanoparticles that can easily be detected by video microscopy. These particles have been shown not to affect the dynamics of the molecules they are attached to, as revealed on model membranes (Mascalchi et al., in preparation), unlike colloidal gold which led to abnormally small diffusion coefficients in previous experiments we carried out on HEK cells (data not shown).

As shown in Fig. 2A, in the conditions used, up to 10 fluorescent spots could be observed simultaneously, each corresponding to individual receptors. The coordinates of each labeled receptor were accumulated over time and compiled as described in the Material and methods section to reconstruct their trajectory (Fig. 2B left). In order to have a complete description of the dynamics of CD4, we only took into account for our analyses the trajectories comprising at least 500 ($x(t)$, $y(t)$) coordinates (i.e. 20 s of duration) with a percentage of the time where QD could not be observed due to blinking inferior to 10%.

3.3. Mobility analysis of CD4 at 20 °C

In order to compare the present results with previous FRAP measurements [5], we first performed SPT experiments at 20 °C. Our results revealed two major distinct behaviors of CD4 on living JCCR5 cells (Table 1). On the one hand, less than half of the receptors (43%) were free to diffuse and exhibited a random Brownian diffusion with an average diffusion coefficient, $D_{1\text{-}2}$, of about 6.10^{-2} μm²/s. On the other hand, the remaining receptors were either transiently (45%) or permanently (12%) confined into ≈220 nm domains. The $D_{1\text{-}2}$ of these confined receptors was nearly the same as that of unconfined receptors (≈5.10^{-2} μm²/s). Worthy of note, when transiently confined diffusion was observed, the segments with random diffusion had similar diffusion coefficients to those measured for the confined ones (≈7.10^{-2} μm²/s). Such a compartmentalization of the diffusion within the membrane has already been observed for many membrane proteins [30–32] and is in agreement with those found for CD4 on transfected HEK cells [5]. Several phenomena could explain this compartmentalization, such as interactions with other membrane proteins [33], lipids [34,35] or the actin cytoskeleton [36] (for review, see [2]). In future work, it will be interesting to explore which factors are responsible for the confinement of the receptor by performing SPT experiments after cholesterol depletion or after perturbation of the organization of the cytoskeleton.