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Mannose-6-Phosphate Receptor, a Novel Checkpoint for T cell Expansion, is expressed at High Levels on T cells from Untreated HIV+ Patients

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Recently, we showed that untreated patients with HIV infection display high peripheral blood counts of regulatory B cells expressing the serine protease granzyme B (GrB) in the absence of perforin (*GraB cells*) [1]. Importantly, these *GraB cells* are able to directly regulate proliferation and survival of T cells both *in-vitro* and *in-vivo*. The mechanism of action involves a perforin-independent transfer of GrBto T cells and GrB-dependent degradation of the T cell receptor ζ-chain in T cells [1,2].

A known receptor for GrB, which acts in a perforin-independent manner, is the mannose-6-phosphate receptor (M6PR, CD222), which has been shown to mediate GrB uptake and regulation of M6PR-expressing target cells [3,4]. A recent study in *Listeria*-infected mice demonstrated that the differential expression of M6PR on cytotoxic T cells is directly linked to their survival and proliferative capacity [5]. M6PR therefore appears to represent an important check point for T cell expansion and memory T cell formation after systemic infections.

Here we report our current findings confirming this mechanism in human patients with untreated HIV infections. Since cellular uptake of GrB in the absence of perforin can occur in an M6PR-dependent manner [3,4], we tested the expression of M6PR on T cells from untreated HIV patients and compared it to healthy controls. These experiments revealed that M6PR expression by T cells from HIV patients is significantly higher than by T cells from healthy control subjects (Figure 1 and Table 1). Moreover, *in-vitro* transfection of isolated T cells from healthy subjects with HIV confirmed that the HIV directly triggers upregulation of M6PR on T cells (Figure 2). Our data therefore suggest that defects in the memory T cell compartment of HIV patients may at least in part be due to elevated expression of

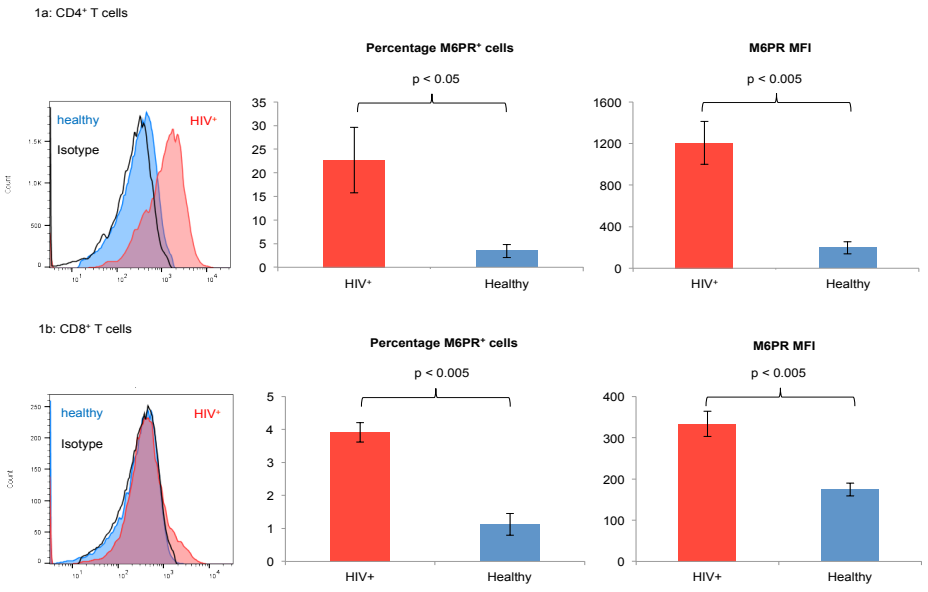

Figure 1: T cells from untreated HIV+ patients express high levels of M6PR as compared with T cells from healthy individuals. PBMC from 4 untreated HIV patients and from 4 healthy control subjects were isolated and stained with fluorescently labeled antibodies against CD3, CD4, CD8 and M6PR (CD222). Subsequently, $CD4^+$ (**Figure 1a**) and $CD8^+$ (**Figure 1b**) T cells were analyzed by flow cytometry. Human peripheral granulocytes served as positive control for CD222 expression. Histograms show M6PR surface expression from one representative experiment out of 4 with similar results (left panels). Bar graphs show average percentages of $M6PR^+$ T cells (middle panels) and M6PR median fluorescence intensity (MFI) values (right panels) from 4 independent experiments. Error bars indicate SEM, *indicates p<0.05, **indicates p<0.005.

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Study Participants	Gender	Age (y)	CD4⁺ T cells/µl	CD4⁺ T cells (%)	HIV-RNA copies/µl	CD222⁺ T cells (%)	CD222⁺ MFI T cells	GrB⁺ B cells (%)	GrB MFI B cells
HIV⁺	M	47	180	8.3	58,400	17.3	1336	2.6	416
HIV⁺	M	37	235	10	713,000	18.3	602	9.9	386
HIV⁺	M	54	67	8.4	1,760,000	12	1362	3.5	277
HIV⁺	M	47	48	4.8	3,170,000	43.1	1524	10.5	384
Healthy	M	42	n.a.	41	n.a.	4.63	109	n.a.	n.a.
Healthy	M	28	n.a.	50	n.a.	0.564	364	n.a.	n.a.
Healthy	F	44	n.a.	57	n.a.	6.68	139	n.a.	n.a.
Healthy	F	59	n.a.	37	n.a.	1.85	171	n.a.	n.a.

Table 1: Clinical, virological, andimmunologicalcharacteristics of HIV patients and healthy control subjects tested for GraB cells and CD222-expressing CD4⁺ T cells.
Abbreviations: F: Female; M: Male; GrB: Granzyme B; MFI: Median fluorescence intensity; n.a., not available.

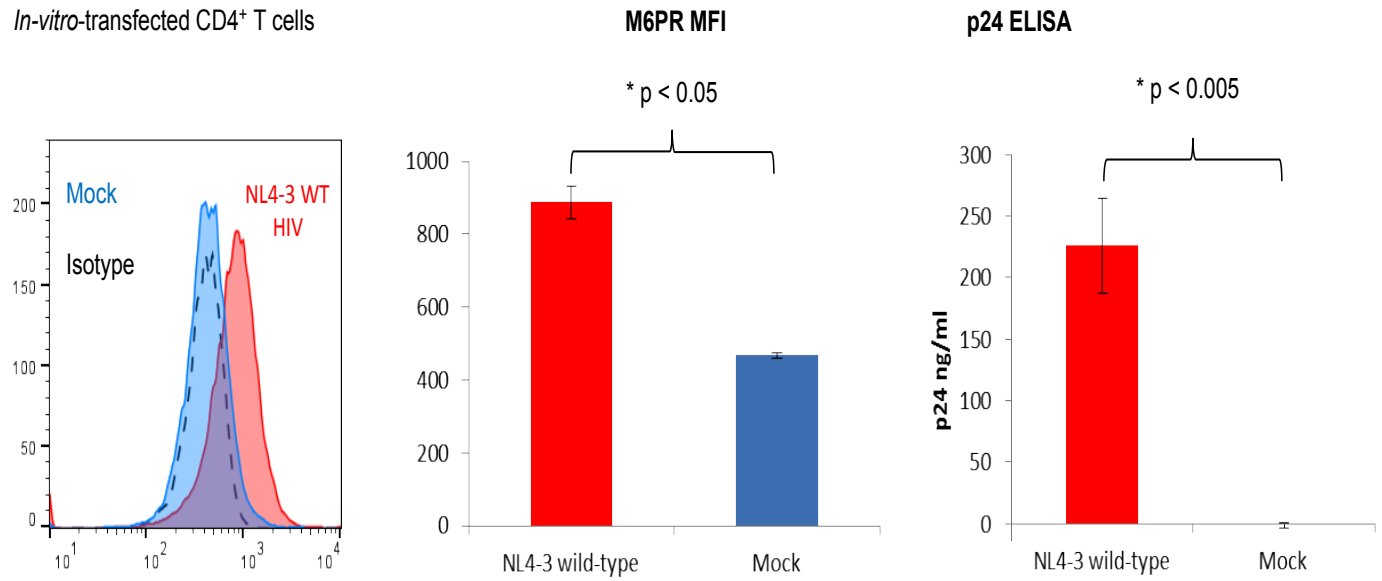

Figure 2: T cells from healthy individuals upregulate mannose-6-phosphate receptor (M6PR) following transfection with wild-type NL4-3 HIV. CD4⁺ T cells from 3 healthy individuals were isolated and stimulated with CD3/CD28 dynabeads and IL-2 for 3 days. Cells were washed and transfected with NL4-3 wild-type (WT) or mock-transfected for 6 hrs at 37°C. Three days post-transfection, cells were stained with fluorescently labeled antibodies against M6PR (CD222) or an isotype control. Then, T cells were analyzed by flow cytometry. Culture supernatants were tested for p24 protein levels using an in-house ELISA (Abcam). Histograms show M6PR surface expression from one representative experiment out of three with similar results (left panel). Bar graphs show M6PR median fluorescence intensity (MFI) values (middle panel) and p24 levels (right panel). Error bars indicate SEM, *indicates p<0.05.

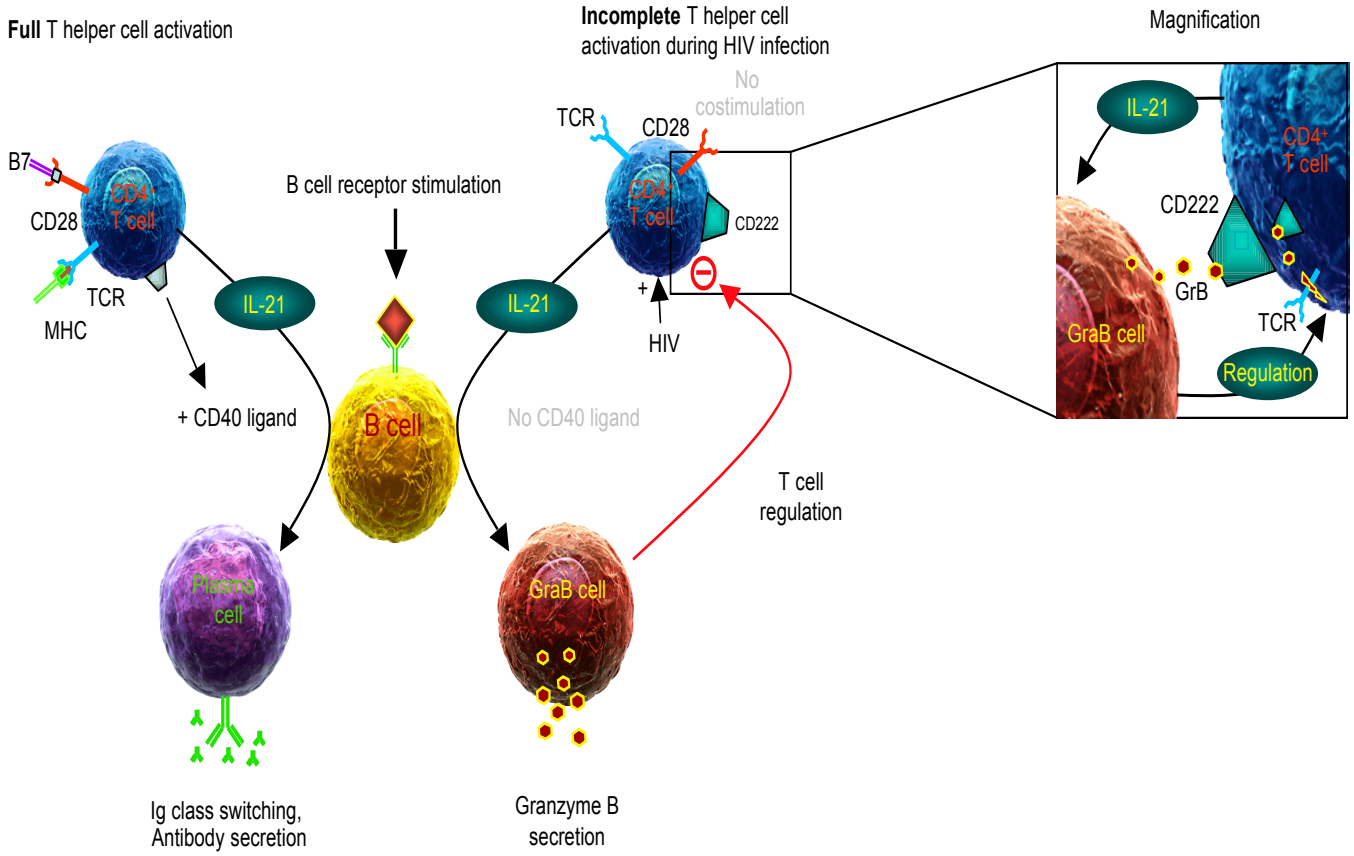

Figure 3: Mannose-6-phosphate receptor (M6PR, CD222) on T cells from HIV patients mediates their suppression by granzyme B-secreting regulatory B cells (*GraB cells*). During HIV infection, the T cell receptor (TCR) of CD4+ T cells is directly stimulated via the HIV protein *Nef*, without simultaneous costimulation of CD28. In contrast to fully activated T cells (left panel side), such incompletely activated T cells secrete IL-21, but barely express CD40L, resulting in the induction of granzyme B-secreting *GraB cells* instead of plasmacells (right panel side, *Copyright 2015. The American Association of Immunologists, Inc.*). By concomitant upregulation of CD222 on T cells in the course of an HIV infection, the cellular uptake of exogenous granzyme B by T cells is strongly enhanced, resulting in increased cleavage of their TCR ζ-chain (magnification panel). Lower TCR ζ-chain levels are associated with lower proliferative capacity of such T cells. Breaking of this vicious circle may be possible by exogenous addition of CD40L multimers, which can suppress the generation of GraB cells after incomplete B cell/T cell interactions during HIV infection.

M6PR by T cells, associated with a higher sensitivity of these cells to GrB-mediated apoptosis and growth arrest.

In summary, our findings support the current view that after infections with intracellular pathogens such as viruses or intracellular bacteria, activated T cells differentially regulate M6PR on their cell surface [5]. This differential M6PR expression may not only explain how regulatory T cells initiate the effector T cell contraction phase after an infection, but also how other immune cell populations expressing GrB in the absence of perforin such as plasmacytoid dendritic cells or GraB cells [2,6,7] may directly suppress T cell expansion in an M6PR- and GrB-dependent fashion (Figure 3). Modulation of M6PR on T cells by pharmacological means may represent a promising novel approach to modulate T cell-mediated immunity in different infectious diseases including HIV infection.

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