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Modulation of CCR5 expression and R5 HIV-1 infection in primary macrophages exposed to sera from HESN, LTNP, and chronically HIV-1 infected people with or without natural antibodies to CCR5

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ABSTRACT

CCR5 is the main co-receptor for HIV-1 cell entry and it plays key roles in HIV-1 mucosal transmission. Natural anti-CCR5 antibodies were found in HIV-1-exposed seronegative and long-term non-progressor subjects, suggesting a role in controlling viral replication *in vivo*. We assessed the effect of sera containing or not natural anti-CCR5 antibodies, on membrane CCR5 level and HIV-1 infection in primary macrophages. Sera modulated CCR5 expression with a trend dependent on the donor/serum tested but independent on the presence or absence of anti-CCR5 antibodies. All sera strongly reduced HIV-1 DNA in all donor's macrophages and no correlation was observed between CCR5 and viral DNA levels. These results suggest that CCR5 expression level is not a major determinant of macrophage infection and that the observed modulation of CCR5 and HIV-1 DNA might depend on factors other than CCR5-reactive antibodies present in sera and/or intrinsic to the donors on which sera were tested.

1. Introduction

CC chemokine receptor 5 (CCR5) is a seven-transmembrane, G protein-coupled receptor (GPCR) that is expressed on T lymphocytes and myeloid cells. Its structure consists of an extracellular N-terminal domain, three extracellular loops (ECL1-3), three intracellular loops, and a cytoplasmic C-terminal tail (Tan et al., 2013). CCR5 functions as the main co-receptor for human immunodeficiency virus type 1 (HIV-1) entry in target cells, and it plays a key role in HIV-1 mucosal transmission, which accounts for more than 95 % of new infections worldwide (Mohamed et al., 2022). The evidence of the crucial role of CCR5 in HIV-1 pathogenesis came from the discovery of the Δ32 allele, a 32 base pair deletion in the CCR5 gene-coding region that gives rise to a non-functional receptor. In humans, homozygosis results in an almost complete protection from HIV-1 infection and is indeed associated with the resistance to infection of some HIV-exposed seronegative (HESN) subjects (Liu et al., 1996). Conversely, heterozygosis determines a reduced CCR5 cell surface expression and is associated with a slower progression toward the disease (Stewart et al., 1997).

Naturally occurring antibodies (Abs) to CCR5 were found in serum as well as in other biological fluids (i.e., saliva, milk, semen, and cervicovaginal secretions) in various groups of individuals. In particular, they were identified in cervicovaginal secretions and breast milk of HIV-seropositive and seronegative women, in normal human IgG for

Abbreviations: CCR5, CC chemokine receptor 5; ECL, extracellular loop; GPCR, G protein-coupled receptor; HESN, HIV-exposed seronegative; LTNP, long-term non-progressor; MDM, monocyte-derived macrophage.

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therapeutic use, in Δ32 homozygous carriers exposed to CCR5 through sexual intercourse with CCR5 wild type partners, and in hemophilic patients repeatedly exposed to alloantigens through continuous blood transfusions (Venuti et al., 2017). Some of these Abs were isolated by affinity chromatography on synthetic peptides corresponding to motifs derived from the HIV-1 binding domains of CCR5 (i.e., N terminus and ECL2). Our group previously characterized Abs directed to CCR5 ECL1 in several cohorts of HESN people in serologically discordant couples and in a subset of HIV-1 seropositive individuals showing long-term infection control in the absence of antiretroviral therapy (long-term non-progressors, LTNPs), suggesting a role for such Abs in natural HIV-1 resistance and in controlling viral replication *in vivo* (Lopalco et al., 2000; Pastori et al., 2006). These Abs do not hamper HIV-1 binding, but they cause a long-lasting internalization of the co-receptor, resulting in inhibition of HIV-1 infection in $CD4^+$ T lymphocytes, T cell lines, and U87.CD4.CCR5 cells and of virus transcytosis through epithelial layers (Barassi et al., 2004; Bomsel et al., 2007; Lopalco et al., 2000; Pastori et al., 2006).

Besides T lymphocytes, macrophages represent a primary target and host of R5- and dual-tropic HIV-1 *in vivo* (Kruize and Kootstra, 2019, Wong et al., 2019). The precise role of these cells in the pathogenesis of HIV-1 infection is yet under debate, however their reduced susceptibility to some antiretroviral drugs and increased resistance to HIV-1-induced cytopathic effect suggest they may contribute to the viral reservoir in the setting of combined antiretroviral therapy (cART) (Hendricks et al., 2021). Studies in humans and animal models have in fact highlighted the involvement of macrophages in viral persistence and in the development of comorbidities associated with HIV-1 and Simian Immunodeficiency Virus infection (Abreu et al., 2019; Abreu et al., 2019a; Abreu et al., 2019b; Arainga et al., 2017; Avalos et al., 2017; Ganor et al., 2019; Honeycutt et al., 2016; Honeycutt et al., 2017; Veenhuis et al., 2023). Compared to $CD4^+$ T lymphocytes, macrophages express low CD4 and CCR5 levels, and are less efficiently infected by cell-free HIV-1 virions, whereas direct cell-cell spread is more efficient (Dupont and Sattentau, 2020). These cells constitutively express antiviral restriction factors that potently reduce HIV-1 replication at post-entry steps of the viral life cycle and their susceptibility to HIV-1 infection is dependent on the local microenvironment, which modulates cell differentiation and polarization (Kruize and Kootstra, 2019, Pagani et al., 2022).

Although some previous studies investigated the effect on macrophage infection of natural Abs targeting CCR5 ECL2 and N terminus (Bouhlal et al., 2005; ; Bouhlal et al., 2001; Eslahpazir et al. 2008), data on the effect of natural Abs directed to CCR5 ECL1 on these cells are lacking. Thus, we herein evaluated the effect of monocyte-derived macrophage (MDM) exposure to sera from HESN, LTNP, chronically HIV-1 infected (HIV-1+), and HIV-1 seronegative individuals (HIV-1-), all characterized for the presence or absence of natural Abs to CCR5 ECL1, on CCR5 membrane expression and HIV-1 infection.

2. Materials and methods

2.1. Ethics statement

Serum samples from healthy blood donors were obtained from Centro Risorse Biologiche, San Raffaele Scientific Institute, Milan. Samples from HESN and HIV-1+ (including LTNP) subjects were obtained from Infectious Diseases Clinic, San Raffaele Scientific Institute, Milan. The Institutional review board named "Comitato Etico della Fondazione San Raffaele del Monte Tabor, Milan, Italy" approved the investigations (Protocol no 95/DG). All subjects provided a written informed consent and all methods were performed in accordance with the relevant Italian guidelines and regulations.

Healthy donor's buffy coats for monocyte isolation were obtained from Centro Trasfusionale, Sapienza University of Rome. Ethical approval was obtained from the Ethic Committee of Istituto Superiore di Sanità (protocol number 0016142). Since data were analyzed anonymously, informed consent was not requested. Healthy donor's data were treated by Centro Trasfusionale conforming to the Italian law on personal data management "Codice in Materia di Protezione dei dati Personali" (Testo unico D.L. June 30, 2003 n. 196).

2.2. Serum samples

Serum samples were obtained from fourteen people (six HESN, two LTNP, two HIV-1+, and four HIV-1- subjects) characterized for the presence/absence of natural anti-CCR5 Abs. Characteristics of the studied populations were reported previously (Lopalco et al., 2000; Pastori et al., 2006). Briefly, the HESN subjects were partners of HIV-1+ individuals discordant for HIV serostatus and had a history of penetrative sexual intercourse without condom for at least two years at least twice per week, with no other known risk factors. The inclusion criteria for the LTNP cohort were (1) seroconversion at least seven years before, (2) absence of antiretroviral therapy, (3) CD4 T cell counts of at least 500 cells/μL, and (4) asymptomatic infection and good health conditions. Control HIV-1+ subjects were on cART and had CD4 counts $\geq$ 500 cells/μL and no previous comorbidities linked to the infection. All patients were screened for CCR5-Δ32. Peripheral venous blood was drawn and serum was separated, heat inactivated, and stored at -80 °C until use.

2.3. Monocyte isolation and differentiation to MDMs

Monocytes were isolated from the peripheral blood of healthy donors and cultured for 5 days to allow differentiation to MDMs as previously reported (Covino et al., 2024). MDMs were screened for the CCR5-Δ32 mutation by using a previously reported primer set (Nischalke et al., 2004).

2.4. HIV-1 infection

The CCR5-dependent HIV-1_{BaL} virus was obtained from the NIH AIDS Research and Reference Pro-gram, Division of AIDS, NIAID, NIH and was propagated on stimulated healthy donor's PBMCs, harvested, and clarified at peak viremia as measured by p24 Ag Elisa Assay (Fujirebio Inc., Tokyo, Japan); virus was stored at -80C°. The 50 % tissue culture infectious dose ($TCID_{50}$) was measured in PHA-stimulated PBMCs at day 7 by HIV p24 antigen quantification using the Spearman-Karber method (National Institute of Allergy and Infectious Diseases (U.S.). Division of AIDS. and National Institutes of Health, (U. S.), 1997). MDMs were treated with different dilutions of sera for 48 hours or with maraviroc (MRV, 10 nM; Sigma-Aldrich) for 1 hour and then infected with 3,000 $TCID_{50}$ per well of the HIV-1_{BaL} virus, corresponding to a MOI $\approx$ 0.03, as previously reported (Covino et al., 2020). After 2 h, cells were washed and maintained in complete medium in the presence or absence of sera.

2.5. Flow cytometry analysis of CCR5 membrane expression

MDMs were detached by using PBS 0.05 % EDTA. Cells were pre-incubated with Fc blocking reagent (Miltenyi Biotec) for 10 min on ice and then stained with a PE-conjugated anti-CCR5 monoclonal Ab (Clone 3A9; BD Biosciences, Milan, Italy) or an isotype-matched Ab (BD Biosciences) to determine the background fluorescence. After 30 min on ice, cells were washed with PBS (Euroclone, Pero, Italy), and acquired with a FACS Calibur flow cytometer by using Cell Quest and the Cell Quest software for analysis (BD Biosciences).

2.6. Quantification of HIV-1 DNA by real-time polymerase chain reaction

Total DNA was extracted from frozen MDM samples as we previously reported (Sabbatucci et al., 2015). DNA concentration was determined by Real Time PCR quantitative amplification of the *RNase P* gene using

TaqMan RNase P Detection Reagents Kit (Applied Biosystems, Monza, Italy) according to manufacturer's instructions. Total HIV-1 DNA amount was determined using primers and probe that recognize the HIV-1 *gag* and *env* genes. Primers and probe for the HIV-1 *gag* gene were previously reported (Cassol et al., 2009; Sabbatucci et al., 2015), whereas those for the HIV-1 *env* gene were designed based on the HIV-1_{BaL} sequence and were as follow: forward primer, 5'-TAGGCAGGGATACTCACCATTA-3'; reverse primer, 5'-GTCTCTCTCTCCACCTTCTTCT-3'; probe, 5'-(FAM) TCGTTTCAGACCCACCTCCCAG (IABkFQ)-3' (Integrated DNA Technologies, Leuven, Belgium). Standard curve was generated using the genomic DNA from the 8E5 cell line, a T lymphoblastoid cell line that contains a single defective copy of HIV-1 genome per cell. All samples and HIV-1 negative controls were run in duplicate. HIV-1 *gag* and *env* and *RNAseP* standard curves had slopes between -3.11 and -3.57 and the coefficient of correlations were > 0.981. The thermal cycling conditions were as follows: holding stage for enzyme activation 95°C for 30 s and amplification with 40 cycles of 95°C for 5 s and 60°C for 30 s. Cycling and data acquisition were carried out using the 7500 Real-Time PCR system (Applied Biosystems).

2.7. Statistical analysis

The non-parametric Spearman test was used to determine correlation coefficients. SPSS version 28.1 (IBM Corp., Armonk, NY, USA) and Excel 2016 (Microsoft, Redmond, WA, USA) were used for statistical analyses and graphs drawing.

3. Results

We studied the outcome of macrophage exposure to sera obtained from three representative HESN (31DDn, 55ASn, and 92CBn) and two representative LTNP (SA40 and SA27) subjects having anti-CCR5 Abs (Table 1). Negative controls were sera from three HESN (81NDn, 42CSn, and 41LAn) and two HIV-1+ cART-treated patients (86AVp and 87CPp) without anti-CCR5 Abs (Table 1). We also included sera obtained from four healthy control subjects (HIV-1-; HC1, NIC, FOR, and SER) characterized for the absence of anti-CCR5 Abs. The effect of sera on CCR5 membrane expression and HIV-1 infection was investigated in MDMs obtained from twelve healthy donors characterized for the absence of the CCR5-Δ32 mutation (Fig. S1). Each serum was tested at a 1:10 (55ASn, 92CBn, SA40, 41LAn, 81NDn, 86AVp, and 87CPp) or at a 1:20 (31DDn, SA27, 42CSn, HC1, NIC, FOR, and SER) dilution in two independent donor's MDMs, with the exception of 31DDn that was evaluated in six different donors and 86AVp and 87CPp that were tested each only in one donor. At least one serum with anti-CCR5 Ab and at least one serum without anti-CCR5 Ab were evaluated in parallel in each donor. After 48 hours of sera exposure, MDMs were infected with HIV-1_{BaL} and the levels of viral DNA were measured at 72-96 hours post-infection by qPCR. MRV was used as a positive control of CCR5 inhibition, and determined a reduction of HIV-1 DNA copies in all the donors, with a range that varied from 70 % to 100 % inhibition (Table S1). CCR5 membrane expression was evaluated by flow cytometry at the time of HIV-1 infection.

Table 1
Demographic and clinical characteristics of HESN, LTNP, and HIV-1^+ subjects.

Anti-CCR5 Ab	Cohort	Subject	Sex	Age	HIV-RNA, copies/mL	$CD4^+$ count, cells/μL
Positive (n = 5)	HESN	31DDn	M	44	n.a.	840
		55ASn	M	34	n.a.	598
		92CBn	M	39	n.a.	656
	LTNP	SA40	M	55	<50	525
		SA27	F	53	<50	732
Median				44	n.a.	656
Negative (n = 5)	HESN	81NDn	M	50	n.a.	576
		42CSn	M	38	n.a.	671
		41LAn	F	41	n.a.	829
	HIV-1^+	86AVp	M	44	10,700	573
		87CPp	M	37	8,500	745
Median				41	n.a.	671
p[a]				0.6	n.a.	1.0

[a] Calculated by Mann-Whitney U test.
n.a., not applicable.

Fig. 1A shows the baseline (in the absence of any treatment) CCR5 levels (blue bars) and viral DNA copies measured by qPCR of *gag* (orange dots) or qPCR of *env* (gray dots). CCR5 was expressed in a low and variable percentage of MDMs (median value 12.6 %, range 3.8-37.1 %; n = 12). Correlation analysis highlighted a strong direct correlation between baseline HIV-1 DNA copies measured by qPCR of *gag* or qPCR of *env* (rs = 0.92, $p < 0.001$) (Fig. 1B), and a lack of correlation between these and baseline CCR5 expression (rs = 0.34, p = 0.28 for *gag*; rs = 0.23, p = 0.47 for *env*) (Fig. S2).

Upon sera exposure, CCR5 expression was modulated in most donors with a trend dependent on the donor/serum tested but independent on the presence (Fig. 2A-B, red bars) or absence (Fig. 2A-B, blue bars) of anti-CCR5 Ab. Of the sera with anti-CCR5 Ab, 55ASn and 92CBn reduced CCR5 expression below 50 % in both donors in which they were tested (55ASn: don3 and don4; 92CBn: don5 and don6), whereas SA40 and SA27 reduced CCR5 levels below 50 % only in one of the two donors in which they were evaluated (SA40: don8; SA27: don9). Furthermore, 31DDn determined a reduction of CCR5 expression in two donors (don11 and don12) and an increase in one donor (don10), whereas it did not modulate CCR5 levels in three donors (don1, don2, and don9) (Fig. 2A and Table S2). Of the sera without anti-CCR5 Abs, HC1 increased CCR5 expression in both donors in which it was tested, whereas the other sera determined a reduction of at least 50 %, with the exception of 41LAn in don3 and NIC in don11 in which there was no modulation (Fig. 2A and Table S2). A modest effect on CCR5 MFI was observed with most of the sera tested (Fig. 2B and Table S2). All sera determined a strong reduction (below 50 %) of viral DNA levels, independently on the presence or absence of anti-CCR5 Ab (Fig. 2C). Correlation analysis highlighted a lack of correlation between the percentage of CCR5 positive cells and viral DNA levels measured in MDMs exposed to either all the different sera (rs = -0.036, p = 0.85) (Fig. S3A) or only to those obtained from the subgroup of HIV-1 negative subjects (rs = -0.336, p = 0.11) (Fig. S3B).

4. Discussion

Here we report a large variation in CCR5 membrane expression and HIV-1 DNA levels in different donor's MDMs and a lack of correlation between these two parameters, suggesting that the levels of infection observed in MDMs are not strictly related to CCR5 expression level. To this regard, it is well known that macrophages derived from CCR5-Δ32 homozygous individuals, which display a complete absence of CCR5 at the cell membrane, are resistant to R5 HIV-1 infection, whereas those derived from CCR5-Δ32 heterozygous donors exhibit a variability in susceptibility to infection similar to that found in cells obtained from wild type individuals (Bol et al., 2009). In agreement with our results, previous studies demonstrated a poor correlation between HIV-1 susceptibility and CCR5 expression in MDMs, except at very low expression levels (Bol et al., 2009; Eisert et al., 2001; Naif et al., 1999; Pesenti et al., 1999). The large variability in the capacity to support HIV-1 replication by different donor's MDMs was attributed to differential expression of host factors that either support or suppress viral replication (Pagani et al., 2022). Furthermore, it was shown that only a fraction of MDMs, which varies in size depending on the blood donor, is able to replicate the virus (Bergamaschi and Pancino, 2010). Thus, it appears that the host factors that regulate HIV-1 replication in MDMs act at the level of both single cells and the individual. The host genetic factors that control MDM permissivity to HIV-1 infection mainly influence pre-reverse transcription steps (Naif et al., 1999). Nevertheless, other steps of the HIV-1 life cycle that can be restricted in MDMs were described. Finally,

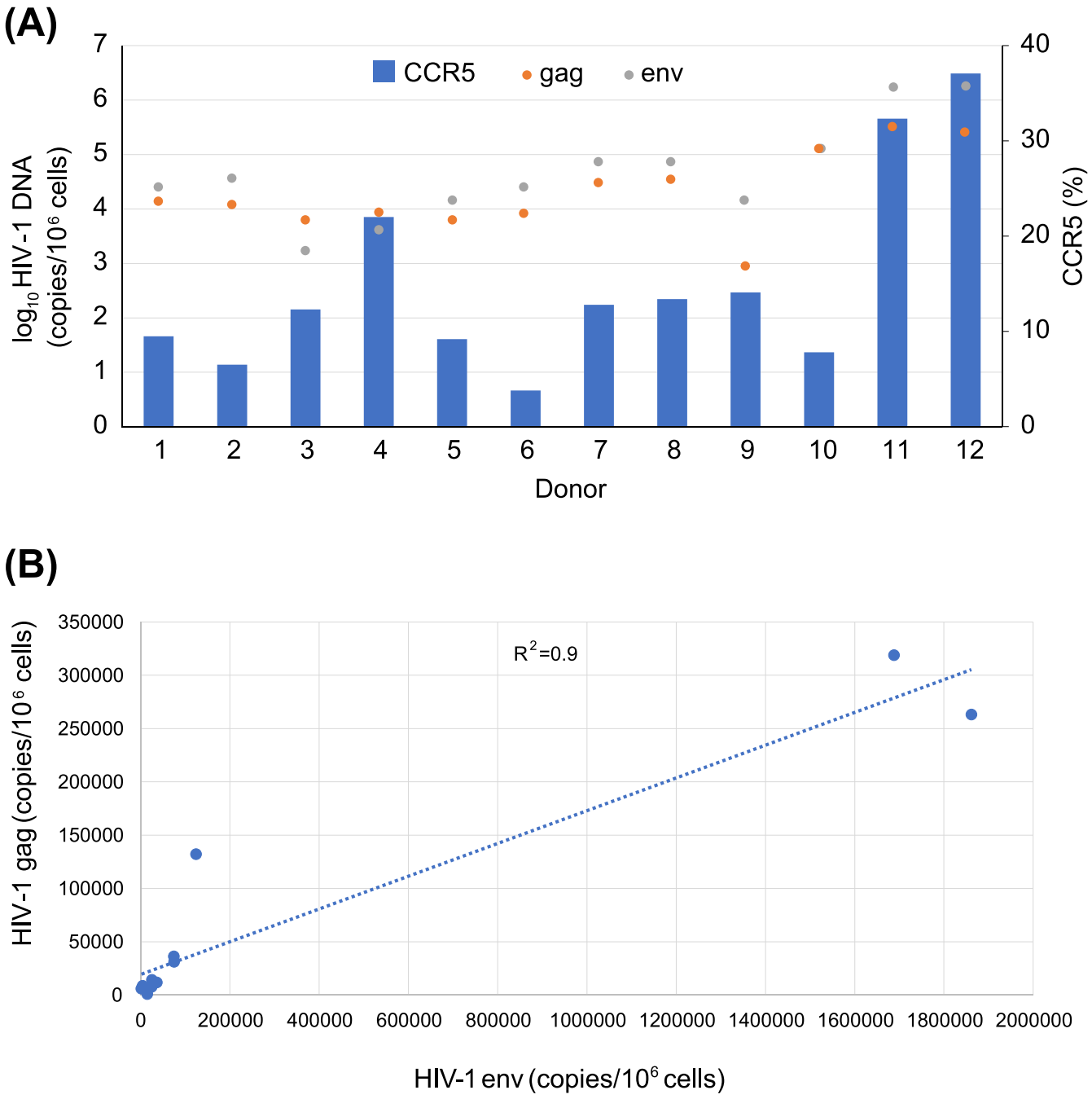

Fig. 1. Baseline CCR5 membrane expression and HIV-1 DNA copies in MDMs. (A) MDMs from twelve healthy donors were infected with R5 HIV-1_{BaL} (3,000 $TCID_{50}$/well). After 72 h (donors 1-6, 9-12) or 96 h (donors 7-8), total DNA was extracted and the amount of total HIV-1 DNA (copies/10^6 cells) was measured by qPCR of *gag* (orange dots) or qPCR of *env* (gray dots). CCR5 membrane expression was assessed at the time of infection by flow cytometry and is shown as percentage (%) of positive cells (blue bars). (B) Correlation between baseline HIV-1 DNA levels measured by qPCR of *gag* and HIV-1 DNA levels measured by qPCR of *env* (n = 12). Statistical analysis was done by using the non-parametric two-tailed Spearman's test to determine correlation coefficient.

besides the CD4/CCR5 mediated entry of HIV-1 into the cell by membrane fusion, alternative routes of infection were identified in macrophages that involves macropinocytosis and virus uptake via the endocytic route (Carter et al., 2011; Marechal et al., 2001).

Natural anti-CCR5 Abs targeting HIV-1 binding domains of CCR5 (i.e., N terminus and ECL2) have been previously shown to hamper infection of MDMs with R5 HIV-1 primary isolates or laboratory-adapted strains (Bouhlal et al., 2005; Bouhlal et al., 2001; Eslahpazir et al. 2008). Interestingly, in one of the reports these anti-CCR5 Abs were found to be more effective at inhibiting the infection of lymphocytes than that of MDMs (Bouhlal et al., 2001). In keeping with these results, the monoclonal Ab PRO-140, which binds an epitope spanning ECL2 and N-terminus, was shown to inhibit MDM infection with different clade A and B viruses, although the median IC_{90} value determined in MDM was six fold higher than the median IC_{90} value observed for the same isolates in PBMC cultures (Trkola et al., 2001). In this study we evaluated for the first time the effect of sera characterized for the presence or absence of natural anti-CCR5Abs directed to ECL1 on CCR5 membrane expression and HIV-1 infection in MDMs. We found that exposure to sera modulated CCR5 expression in most donor-derived MDMs, with a trend dependent on the donor/serum pair but independent on the presence or absence of anti-CCR5 Abs. As we reported previously, the range of anti-CCR5 Ab

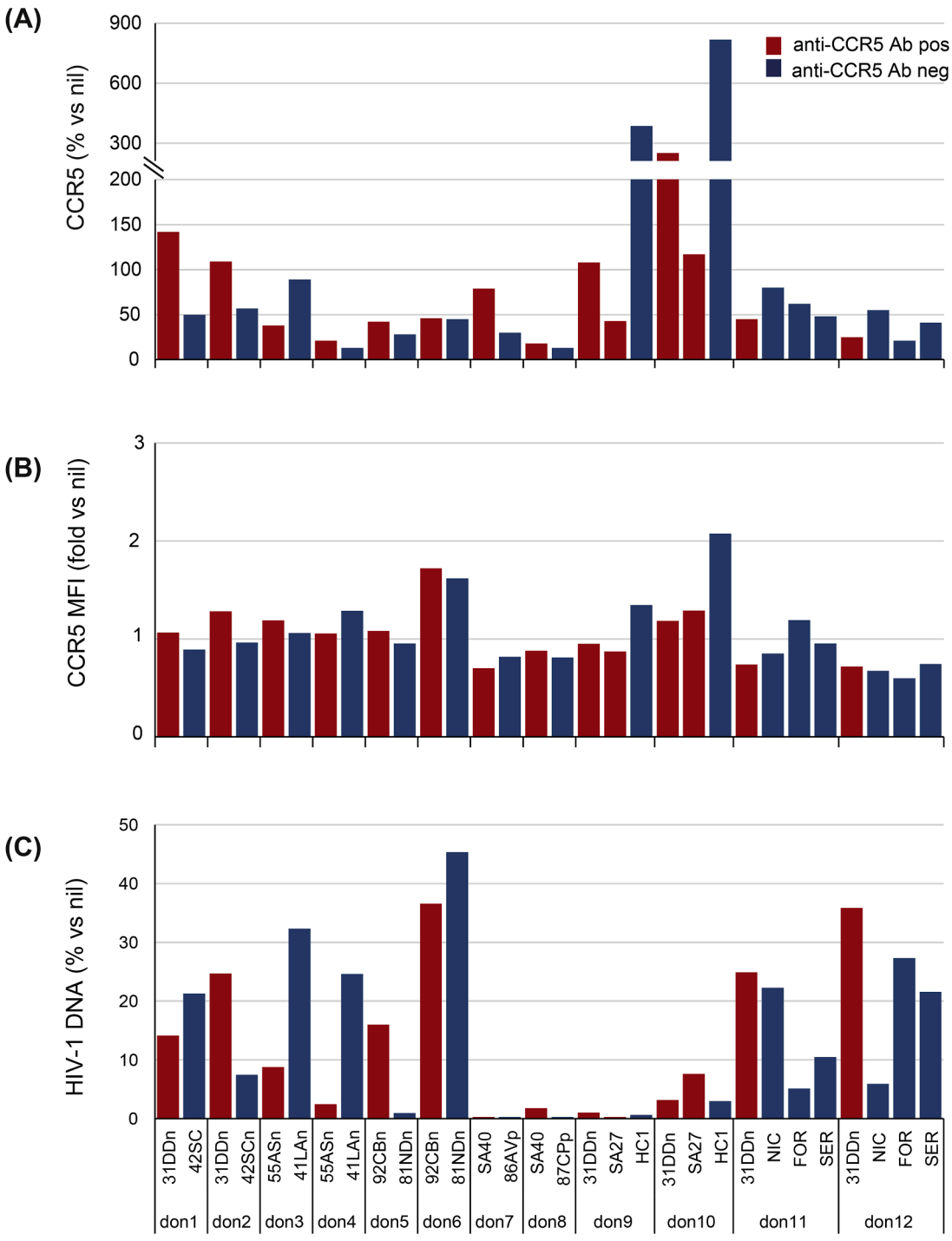

Fig. 2. Effect of sera on membrane CCR5 expression and HIV-1 DNA copies in MDMs. MDMs were exposed to sera from five subjects with anti-CCR5 Abs [three HESN (31DDn, 55ASn, and 92CBn) and two LTNP (SA40 and SA27)] (red bars), and nine subjects without anti-CCR5 Ab [three HESN (42CSn, 41LAn, and 81NDn), two HIV-1+ (86AVp and 87CPp), and four HIV-1- (HC1, NIC, FOR, and SER)] (blue bars). Each serum was evaluated in one (86AVp and 87CPp), two (55ASn, 92CBn, SA40, SA27, 41LAn, 81NDn, 42CSn, HC1, NIC, FOR, and SER), or six (31DDn) donors. Sera were employed at 1:10 (55ASn, 92CBn, SA40, 41LAn, 81NDn, 86AVp, and 87CPp) or 1:20 (31DDn, SA27, 42CSn, HC1, NIC, FOR, and SER) dilution. (A and B) After 48 hours, membrane CCR5 expression was evaluated by flow cytometry. CCR5 expression is shown as either percentage change versus untreated cells (% vs nil) (A) or mean fluorescence intensity (MFI) fold change versus untreated cells (fold vs nil) (B). (C) After 48 hours, cells were infected with R5 HIV-1_{BaL} (3,000 $TCID_{50}$/well). After 72 h (donors 1-6, 9-12) or 96 h (donors 7-8), total DNA was extracted and the amount of total HIV-1 DNA (copies/10^6 cells) was measured by qPCR of *env*. HIV-1 DNA is shown as percentage versus untreated cells (% vs nil). Data represent mean value (+SD) of duplicate measure of HIV-1 DNA copies/10^6 cells. In C, values corresponding to don7/86AVp and don8/87CPp are not visible in the graph because they were very low (< 0.2).

concentrations detected in different serum samples was quite narrow (Pastori et al., 2006), thus it is unlikely that variability in Ab amount may explain the different activities of the sera with anti-CCR5 Abs. Conversely, a strong reduction of R5 HIV-1 DNA levels was observed in all the donor/serum pairs tested. These results are in contrast to those previously obtained in $CD4^+$ T lymphocytes, where only sera with anti-CCR5 Abs determined CCR5 internalization and inhibition of R5 HIV-1 infection (Lopalco et al., 2000, Pastori et al., 2006). These differential effects could be explained at least in part by cell type-specific sensitivity of the receptor to internalization, which depends on CCR5 conformational heterogeneity. To this regard, it was shown that GPCRs can adopt various conformational states based on interactions with signaling ligands, the membrane environment, and specific cellular proteins (Park, 2012). Previous studies using chemokines, Abs, and HIV-1 glycoproteins demonstrated that CCR5 can exist in different cell surface conformational states, each with distinct ligand binding properties (Colin et al., 2013; Colin et al., 2018; Fox et al., 2015; Scurci et al., 2021). Several factors have been proposed to explain the existence of these cell surface subpopulations, such as receptor oligomerization state, receptor G-protein coupling state, local differences in plasma membrane lipid composition, and the level of cell surface CCR5 sulfation (Colin et al., 2013; Colin et al., 2018; Fox et al., 2015; Scurci et al., 2021). Interestingly, conformationally different CCR5 subpopulations associated with differential capacity to bind diverse gp120s and to support viral entry, were suggested as a possible explanation of the poor protection from HIV-1 infection exerted by chemokines in macrophages compared to T lymphocytes (Colin et al., 2018; Fox et al., 2015).

In macrophages, both CCR5 expression and HIV-1 infection/replication are profoundly modulated by the cytokine/chemokine network (Kedzierska et al., 2003). Thus, cytokines and/or chemokines present in sera could explain the observed effects on CCR5 expression and viral DNA levels. The effect of cytokines/chemokines on HIV-1 replication in macrophages can be inhibitory (e.g., type I IFNs and β-chemokines) as well as enhancing (e.g., M-CSF and IL-6) or bi-functional (e.g., IFN-γ and TNF-α), depending on the state of the macrophages at the time of infection and on treatment modalities. The inhibitory factors can restrict viral replication at both entry and post-entry levels of the viral replication cycle. Some studies reported an inverse correlation between the effect on HIV-1 replication and that on CD4 and/or CCR5 expression (Wang et al., 2002), suggesting that factors other than CCR5 and/or CD4 expression levels are involved in the pathways that regulate viral replication in MDMs exposed to certain cytokines/chemokines. For instance, inhibition of HIV-1 replication following macrophage exposure to some cytokines (e.g., type I IFNs) was associated with induced expression of cellular antiviral proteins and anti-HIV miRNAs (Cobos Jimenez et al., 2012).

5. Conclusions

In conclusion, our results suggest that CCR5 membrane expression is not a major determinant of infection levels in MDMs and that the observed modulation of CCR5 and HIV-1 DNA may depend on factors other than CCR5-reactive Abs present in sera and/or intrinsic to the donors on which sera were tested. Main limitations of our study are the small number and heterogeneity of sera analyzed, the possible presence of interfering factors in some serum samples (e.g., anti-HIV Ab or residual drugs respectively in LTNP or HIV-1+ samples), and the lack of molecular characterization of sera (due to the low amount of serum available). Nevertheless, this study adds a small piece of evidence of the distinct features of HIV-1 infection regulation in macrophages and T lymphocytes, and further highlights the notion that results obtained from experiments involving T lymphocytes cannot be extrapolated to macrophages. Future studies are needed to clarify the nature of the serum factor/s responsible for the observed modulation of CCR5 expression and HIV-1 DNA levels in MDMs, as well as the role of natural anti-CCR5 Abs on macrophage HIV-1 infection *in vivo*.

CRediT authorship contribution statement

Iole Farina: Writing – review & editing, Investigation, Conceptualization. **Mauro Andreotti:** Writing – review & editing, Investigation, Conceptualization. **Claudia Pastori:** Resources, Investigation. **Roberta Bona:** Writing – review & editing, Investigation. **Clementina Maria Galluzzo:** Investigation. **Roberta Amici:** Investigation. **Cristina Purificato:** Investigation. **Caterina Uberti-Foppa:** Resources. **Agostino Riva:** Resources. **Maria Cristina Gauzzi:** Writing – review & editing. **Lucia Lopalco:** Writing – review & editing, Resources. **Laura Fantuzzi:** Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2024.199506.

Data availability

Data will be made available on request.

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