MAJOR ARTICLE

Human Immunodeficiency Virus (HIV)-Negative Men Who Have Sex With Men Have Higher CD8+ T-Cell Counts and Lower CD4+/CD8+ T-Cell Ratios Compared With HIV-Negative Heterosexual Men

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Background. We previously reported T-cell senescence to be similar in people with human immunodeficiency virus (PWH) with suppressed viremia (predominantly men who have sex with men [MSM]) and human immunodeficiency virus (HIV)-negative otherwise comparable controls but greater than in healthy blood donors. This led us to compare CD4+ and CD8+ T-cell counts and CD4+/CD8+ ratios between HIV-negative MSM and men who only have sex with women (MSW) and relate observed differences in behavioral factors and infectious exposures, including cytomegalovirus (CMV) infection.

Methods. In 368 HIV-negative MSM and 72 HIV-negative MSW, T lymphocyte phenotyping was performed 3 times biennially. Baseline CMV serology and sexually transmitted infection (STI) incidence and/or STI seroprevalence, sexual, and substance-use behavior data were collected during study visits.

Results. Men who have sex with men, compared with MSW, had higher CD8+ counts (551 vs 437 cells/mm^3, $P < .001$), similar CD4+ counts (864 vs 880 cells/mm^3, $P = .5$), and lower CD4+/CD8+ ratios (1.84 vs 2.47, $P < .001$). Differences were most pronounced for MSM with >10 recent sex partners and partly explained by higher CMV seroprevalence in MSM.

Conclusions. These findings suggest that factors other than HIV may, in both PWH and certain HIV-negative MSM, contribute to a low CD4+/CD8+ ratio. Whether this, like in PWH, contributes to comorbidity risk in HIV-negative MSM requires further study.

Keywords. CD4-CD8 ratio; cytomegalovirus; HIV; men who have sex with men; sexual and gender minorities.

Since the beginning of the human immunodeficiency virus (HIV) epidemic, CD4+ (CD4) and CD8+ T lymphocyte (CD8) counts and the CD4/CD8 ratio have been studied as potential predictive markers of disease progression in people with HIV (PWH). We now know that CD4 count restoration after combination antiretroviral therapy (cART) initiation is strongly correlated not only with a lower likelihood of developing

acquired immune deficiency syndrome (AIDS), but also non-AIDS-associated comorbidities [1, 2]. Furthermore, although evidence remains inconclusive [3], studies have suggested a low CD4/CD8 ratio during long-term cART to be predictive for the development of non-AIDS-associated comorbidities [4–9]. People with HIV remain at increased risk of such comorbidities despite sustained viral suppression on cART [10, 11]. In HIV-negative populations, a low CD4/CD8 ratio with an expansion of CD8+ T cells that express CD57 or lack expression of CD28 has been described as reflecting part of an "immune risk pheno-type" [12, 13]. Thus, low CD4/CD8 ratios, in both HIV-positive and HIV-negative populations, have been associated with mor-bidity [14] and mortality [15–17]. An in-depth analysis of T-cell senescence and activation was performed on a limited sample of AGE$_h$IV participants [18] and in those enrolled in the COBRA (COmorBidity in Relation to AIDS) study linked to it [19]. Both cohorts include an HIV-negative control group, with a majority of men who have sex with men (MSM), to ensure good com-parability with the HIV-positive index group. A second con-trol group of blood bank donors was only included within the COBRA cohort, who had low risk of bloodborne infectious

diseases. CD4$^+$ and CD8$^+$ T-cell senescence, as measured by the expression of CD57 or loss of CD27 and CD28 expression, was comparable between HIV-positive and HIV-negative participants but higher for both groups when compared with blood bank donors. This result suggests increased T-cell senescence also in HIV-negative COBRA/AGE$_h$IV participants. Cytomegalovirus (CMV) seropositivity was an important driver of this increased T-cell senescence in both HIV-positive and HIV-negative COBRA participants. Likewise, CMV seropositivity has also been associated with high CD8 counts and low CD4/CD8 ratios in PWH [20].

In many countries, including the Netherlands, the majority of PWH are MSM [21]. Men who have sex with men are not only at increased risk of acquiring HIV but also of acquiring a wide range of other sexually transmitted infections (STIs). These not only include treatable bacterial STIs such as syphilis, gonorrhea, and chlamydia but also chronic viral infections such as viral hepatitis and herpesvirus infections, including CMV [22]. Cytomegalovirus seropositivity in particular [23], but also transient bacterial STIs [24, 25], have each been associated with a decreased CD4/CD8 ratio and an increased CD8 count.

In view of our earlier observation, we evaluated CD4 and CD8 counts and CD4/CD8 ratios in a larger group of HIV-negative individuals, comparing MSM and men who only have sex with women (MSW). We evaluated to what extent any observed differences were related to sexual and behavioral factors, exposure to STIs, and CMV infection.

METHODS

Study Population

The prospective AGE$_h$IV Cohort Study investigates age-related comorbidities and their risk factors in HIV-positive and HIV-negative individuals aged ≥45 years [10]. Human immunodeficiency virus-positive participants were recruited from the Academic Medical Center's ambulatory HIV clinic, and HIV-negative participants were recruited from the sexual health clinic and the Amsterdam Cohort Studies on HIV/AIDS at the Public Health Service of Amsterdam [26]. Participants undergo standardized biennial screening to evaluate the presence of, or risk factors for, age-associated comorbidities. Most participants in the HIV-negative cohort are MSM, with a limited number of MSW and women. For the current analysis, only HIV-negative men with data available on sexual behavior were included (Figure 1). Human immunodeficiency virus-positive participants and women were not included to allow evaluation of the effects of being an MSM independent from the effects of gender and HIV. Participants with HIV seroconversion during follow-up were excluded from the time of seroconversion onward. All participants provided written informed consent; the study protocol was approved by the local ethics review committee and is registered at www.clinicaltrials.gov (identifier NCT01466582).
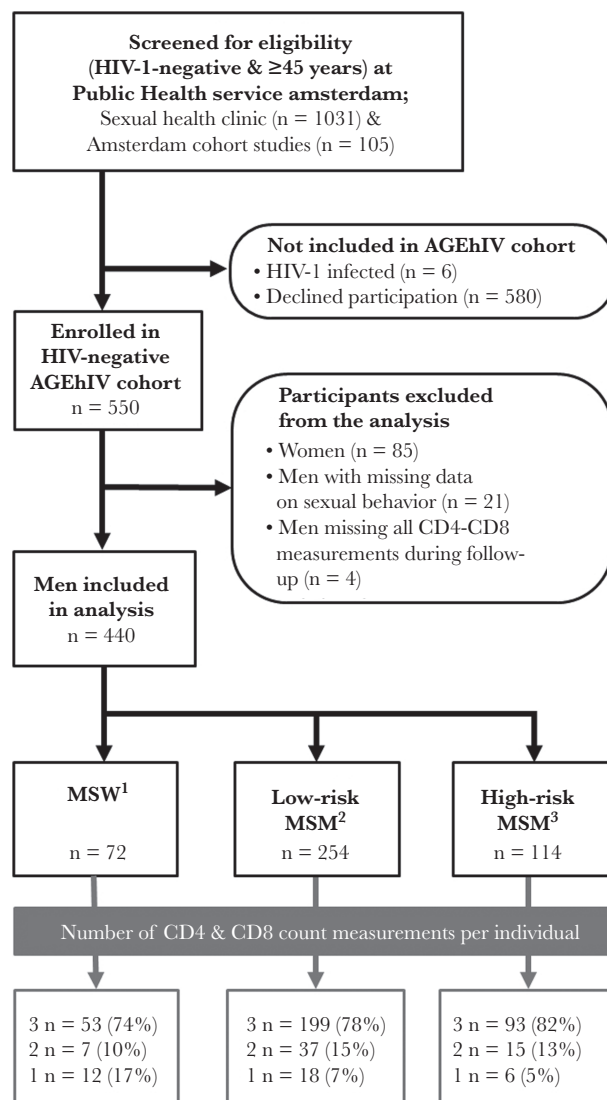
Figure 1. Flow diagram of included participants and measurements. [1]MSW = men who have sex with women and reported <2 male sex partners in their lifetime. [2]Low-risk MSM = men with ≥2 male sex partner in their lifetime and on average ≤10 sex partners during 6 months before study visits; includes 1 male-to-female transgender individual. [3]High-risk MSM = men with ≥2 male sex partner in their lifetime and on average >10 sex partners during 6 months before study visits.

Measurements

Data from baseline and 2 follow-up study visits were available for analysis, comprising 4 years of follow-up. During each study visit, information about sexual behavior, smoking, alcohol use, and recreational drug use were obtained through standardized questionnaires inquiring about lifetime exposure and exposure during the 6 months before the study visits. In addition, during each study visit, percentages and absolute counts of T, B, and natural killer (NK) cells and CD4 and CD8 subpopulations of T cells in peripheral blood were determined using BD Multitest 6-color TBNK reagent with BD Trucount Tubes (BD Biosciences) by flow cytometry (BD FACSCanto II; BD Biosciences). Serology for HIV (4th-generation antibody/

antigen test) and syphilis (*Treponema pallidum* particle agglutination assay and rapid plasma reagin) were also performed at each visit. Chronic hepatitis B (hepatitis B surface antigen positive) and hepatitis C (hepatitis C virus ribonucleic acid [RNA] positive) statuses were determined only at baseline. In stored baseline plasma samples, CMV-immunoglobulin (Ig)G titer (ELISA-VIDITEST; VIDIA, Praha, Czech Republic) and levels of intestinal fatty acid binding protein ([I-FABP] a biomarker for intestinal integrity), soluble (s)CD14, sCD163 (markers of monocyte activation), and interleukin (IL)-6 (marker of inflammation) were determined using enzyme-linked immunosorbent assays (ELISAs) (DuoSet ELISA; R&D Systems). Data on syphilis, chlamydia, and gonorrhea diagnosed at the municipal sexual health clinic in between study visits were extracted from clinical records. For a subset of participants (n = 92) (Supplementary Table 1.1), data regarding percentages of activated ($CD38^+$/HLA-DR^+) and senescent ($CD27^-$/$CD28^-$) T-cells within total CD4 and CD8 T-cell populations were available from analyses we previously published [18, 19].

Definitions

Men who have sex with men was defined as men who reported having had ≥2 male lifetime sex partners, and MSW was defined as men with <2 lifetime male sex partners, given that repeated exposures were the primary focus of interest. After finding large differences in CD4/CD8 ratios between MSW and MSM, but also between MSM with high versus low numbers of recent sex partners in a preliminary analysis, we decided, for this analysis, to further stratify MSM into high- and low-risk categories for the exploration of potential factors driving differences between study groups. High-risk as opposed to low-risk MSM were defined as MSM who reported a mean of >10 vs ≤10 sex partners in the 6 months before each of the 3 study visits, respectively, using the number of sex partners as proxy for the risk of both STIs and HIV.

Statistical Analysis

Characteristics of MSW, low-risk MSM, and high-risk MSM were compared using Wilcoxon rank-sum and χ^2 tests as appropriate. Differences in $\log_e$-transformed CD4/CD8 ratios and $\log_e$-transformed CD4 and CD8 counts between study groups were determined using linear mixed-effects models to account for repeated measurements. Models included a random intercept and random slope for time since baseline for each participant. We subsequently included potential confounding and/or mediating factors in the models following a stepwise forward variable selection process to evaluate which factors were driving any observed differences in lymphocyte subsets between subgroups. The variables body mass index (BMI), recent gonorrhea diagnosis, recent chlamydia diagnosis, and those concerning sexual and substance use behaviors were time updated. To allow differentiating between effects caused by different behavioral patterns between participants

(between-participant variation) versus changes in an individual's behavior over time (within-participant variation), we used a technique called "within subject centering" [27]. This means continuous predictor variables were split into 2. One dummy variable was fixed in time and consisted of the participant's calculated mean of that variable over the 3 study visits, for the analysis of between-participant variation. A second dummy variable consisted of the deviation from the individual's mean at a particular study visit, for the analysis of within-participant variation. Because the covariates CMV seropositivity, HCV RNA status, and I-FABP levels were only measured at the baseline visit, we also performed sensitivity analyses using baseline-data only. Additional mixed-effects models were built to evaluate associations of activation and senescence markers with absolute T-cell counts and the mean differences of these markers among MSW, low-risk MSM, and high-risk MSM, for the subgroup of participants in whom these markers were available [18, 19].

RESULTS

Differences in Participant Characteristics

Seventy-two MSW, 254 low-risk MSM, and 114 high-risk MSM were included in this analysis, 74%, 78%, and 82% of whom, respectively, completed 4 years of follow-up (Figure 1). High-risk MSM were slightly younger than low-risk MSM (Table 1). Men who have sex with men generally were more highly educated, used recreational drugs more often, and smoked less frequently compared with MSW. Participants' estimated lifetime number of sex partners differed substantially: MSW had a median of 25 (interquartile range [IQR], 15–50), compared with 200 (IQR, 50–700) and 1000 (IQR, 500–2000) estimated lifetime sex partners in low-risk MSM and high-risk MSM, respectively. All STI rates were higher in MSM, especially in high-risk MSM; however, chronic hepatitis B and C were rare (prevalence <2% for both in all groups). Baseline CMV seroprevalence was higher in low-risk MSM compared with MSW (76% vs 54%, $P < .001$) and highest in high-risk MSM (90%, $P_{\text{high-risk MSM vs low-risk MSM}} = .003$). During follow-up, none of the MSW but 2 low-risk MSM and 4 high-risk MSM seroconverted for HIV (incidence rate 0, 2.1, and 9.3 per 1000 person-years of follow-up, respectively).

Higher CD8 Counts and Lower CD4/CD8 Ratios in Men Who Have Sex With Men (MSM), Especially in High-Risk MSM

Including all measurements from all 3 study visits, MSM, compared with MSW, had higher mean CD8 counts (551 vs 437 cells/mm^3, $P < .001$), similar mean CD4 counts (864 vs 880 cells/mm^3, $P = .5$), and lower mean CD4/CD8 ratios (1.84 vs 2.47, $P < .001$), respectively. Furthermore, high-risk compared with low-risk MSM had higher mean CD8 counts (599 vs 529 cells/mm^3, $P = .013$), similar mean CD4 counts (824 vs 881, $P = .097$), and mean lower CD4/CD8 ratios (1.59 vs 1.96, $P < .001$), respectively. We calculated the within-subject mean CD4 count, mean CD8 count, and mean CD4/CD8 ratio, for each participant separately,

Table 1. Baseline Characteristics of MSM and Low-Risk and High-Risk MSM

Characteristics	MSW	Low-Risk MSM (≤10 Partners)	High-Risk MSM (>10 Partners)	P Value MSW vs Low-Risk MSM[a]	P Value Low-Risk MSM vs High-Risk MSM[a]
N	72	254	114		
Age in years	52 (48–60)	53 (48–59)	51 (47–56)	1	.02
BMI (kg/m²)	25 (24–29)	25 (23–27)	24 (23–26)	.009	.06
Born in the Netherlands	55 (76.4%)	218 (86.5%)	93 (82.3%)	.04	.3
High educational attainment[b]	32 (45.1%)	157 (62.5%)	70 (64.2%)	.008	.8
Estimated[c] number of lifetime male sex partners	0 (0–0)	200 (50–700)	1000 (500–2000)	<.001	<.001
Estimated[c] number of lifetime female sex partners	25 (15–50)	2 (0–5)	2 (0–5)	<.001	.7
Number of male sex partners prior 6 months	0 (0–0)	2 (1–5)	20 (10–30)	<.001	<.001
Number of recent female sex partners prior 6 months	2 (1–3)	0 (0–0)	0 (0–0)	<.001	.5
Tobacco smoking during last month	25 (34.7%)	56 (22.2%)	22 (19.5%)	.03	.6
Heavy daily drinking[d,e]	7 (9.7%)	12 (4.8%)	6 (5.4%)	.1	.8
Recreational drug use[e]	11 (15.5%)	68 (27.1%)	47 (41.6%)	.05	.006
Daily marijuana use[e]	4 (5.6%)	11 (4.4%)	2 (1.8%)	.7	.2
Use of MDMA, monthly or more frequent[e]	3 (4.2%)	18 (7.2%)	14 (12.4%)	.4	.1
Neisseria gonorrhoeae diagnosis at recruitment visit	1 (1.4%)	9 (3.6%)	11 (9.6%)	.4	.02
Chlamydia trachomatis diagnosis at recruitment visit	2 (2.8%)	17 (6.7%)	10 (8.8%)	.2	.5
Ever contracted syphilis[f]	1 (1.4%)	53 (20.9%)	32 (28.1%)	<.001	.1
Syphilis diagnosis at recruitment visit[g]	1 (1.4%)	2 (0.8%)	5 (4.4%)	.6	.02
HCV RNA positive	0 (0.0%)	2 (0.8%)	2 (1.8%)	.5	.4
HBsAg positive	0 (0.0%)	1 (0.4%)	2 (1.8%)	.6	.2
CMV IgG-seropositive	39 (54.2%)	193 (76.3%)	102 (89.5%)	<.001	.003

Abbreviations: BMI, body mass index; CMV, cytomegalovirus; HbsAg, hepatitis B surface antigen; HCV, hepatitis C virus; IgG, immunoglobulin G; MDMA, 3,4-methylenedioxymethamphetamine; MSM, men who have sex with men; MSW, men who only have sex with women; RNA, ribonucleic acid.

NOTE: Given values are median (interquartile range) or n (%).

[a]χ² tests were used for comparison, except for age, BMI, and numbers of sex partners where Wilcoxon rank-sum tests were used.

[b]Finished vocational level education or higher.
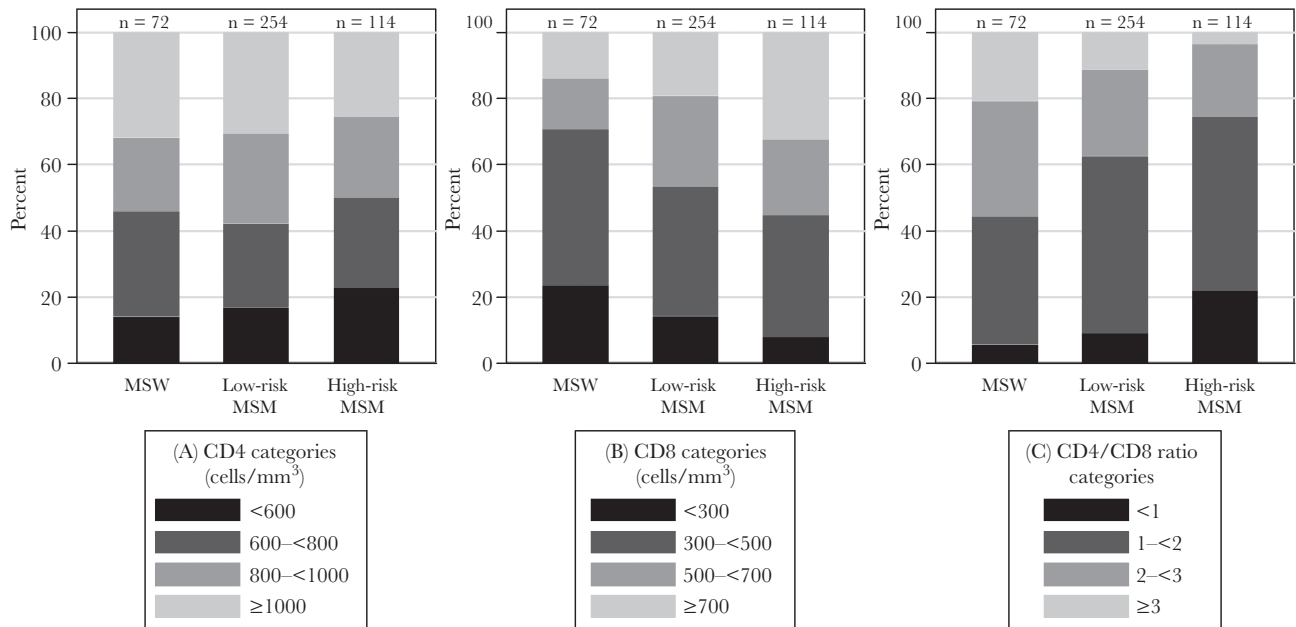
[c]Estimated by participant himself.

[d]Drinking > 4 international units of alcohol daily or almost daily.

[e]During prior 6 months.

[f]*Treponema pallidum* particle agglutination assay positive.

[g]Rapid plasma reagin >1:4.

Figure 2. Distribution of per participant mean absolute CD4 and CD8 counts and mean CD4/CD8 ratios stratified by risk group. MSM, men who have sex with men; MSW, men who only have sex with women.

based on all his available measurements (from at most 3 study visits). Figure 2 shows the distribution of these mean CD4/CD8 ratios and mean CD4 and CD8 counts, stratified by MSW, low-risk MSM, and high-risk MSM. Mean CD8 counts were higher and mean CD4/CD8 ratios lower with higher numbers of recent sex partners, with 5.6% of MSW having a mean CD4/CD8 ratio

below 1, as opposed to 9.1% of low-risk MSM and 21.9% of high-risk MSM. A CD4/CD8 ratio <.5, observed at just a single study visit, was found in 4 (2%) low-risk MSM, 1 (1%) high-risk MSM, and no (0%) MSW. The cutoff for having on average >10 recent sex partners (ie, "high-risk") was derived from the analysis shown in Table 2. Three separate multivariable linear mixed-effects

Table 2. Associations of Being MSM and the Reported Number of Sex Partners in the 6 Months Before Study Visits With the Absolute CD4 and CD8 Counts and CD4/CD8 Ratio[a]

Variable	N[b]	$\log_e$(CD4)		$\log_e$(CD8)		$\log_e$(CD4/CD8)	
		Coeff	95% CI	Coeff	95% CI	Coeff	95% CI
<2 lifetime male-male sexual contact (MSW)	181	ref	ref	ref	ref	ref	ref
≥2 lifetime male-male sexual contact (MSM)	968	−0.01	−0.10 to 0.07	0.19**	0.06 to 0.31	−0.20**	−0.32 to −0.08
Mean number of sex partners during 6 months before study visits, per participant (between participant variation)							
≤2 sex partners	410	ref	ref	ref	ref	ref	ref
>2 to ≤10 sex partners	432	−0.01	−0.09 to 0.06	0.02	−0.08 to 0.12	−0.03	−0.13 to 0.07
>10 to ≤30 sex partners	225	−0.05	−0.14 to 0.04	0.14*	0.02 to 0.27	−0.19**	−0.31 to −0.07
>30 sex partners	82	−0.08	−0.21 to 0.05	0.14	−0.05 to 0.32	−0.21*	−0.39 to −0.04
Deviation from individual's mean number of sex partners reported at current visit (within participant variation)							
≤5 sex partners higher or lower	931	ref	ref	ref	ref	ref	ref
>5 sex partners higher	105	−0.02	−0.07 to 0.03	−0.01	−0.08 to 0.05	−0.00	−0.05 to 0.04
>5 sex partners lower	113	−0.02	−0.07 to 0.03	−0.02	−0.09 to 0.05	0.00	−0.04 to 0.05

Abbreviations: CI, confidence interval; Coeff, coefficient; MSM, men who have sex with men; MSW, men who only have sex with women; ref, reference category.

[a]Results of multivariable linear mixed-effects models.

[b]Number of measurements per variable category. Estimates are derived from 3 multivariable linear mixed-effects models with the $\log_e$ transformed absolute CD4 and CD8 counts and $\log_e$ transformed CD4/CD8 ratio as outcome variables. The models include a per participant random intercept and random slope for time since baseline, using an unstructured covariance matrix. Models also included adjustment for time since baseline and time in the day of blood draw.

*$P < .05$.

**$P < .01$.

models were constructed to analyze predictors of CD4 count, CD8 count, and CD4/CD8 ratio. In addition to 2 potential confounding variables (time since baseline and time in the day of blood draw), the models include the following 3 predictor variables of interest: (1) MSW vs MSM; (2) mean number of recent sex partners over all study visits of the participant; and (3) variation in number of recent sex partners between the mean and the actual visit. Being MSM and having either >10 or >30 recent sex partners "on average during study follow-up", each were independently associated with higher CD8 counts ($P_{\text{MSM vs MSW}}$ .003, $P_{>10 \text{ vs} \leq 2 \text{ partners}}$ .028, $P_{>30 \text{ vs} \leq 2 \text{ partners}}$ .14) and lower CD4/CD8 ratios ($P_{\text{MSM vs MSW}}$ .001, $P_{>10 \text{ vs} \leq 2 \text{ partners}}$ .003, $P_{>30 \text{ vs} \leq 2 \text{ partners}}$ .017). Changes in an individual's number of recent sex partners during study follow-up were not associated with differences in CD4 or CD8 counts.

Lower CD4/CD8 Ratio for a Large Part Explained by Higher Cytomegalovirus-Seroprevalence in Men Who Have Sex With Men

Figure 3 shows results of multivariable models adjusted both for potential confounders and mediators of the differences among MSW, low-risk MSM, and high-risk MSM. The differences in CD4/CD8 ratios and absolute CD8 counts between groups were reduced after addition of baseline CMV serostatus to the models, with the difference in CD4/CD8 ratio between MSW and low-risk MSM remaining borderline statistically significant ($P = .051$). The differences in CD4/CD8 ratios between high-risk MSM and both low-risk MSM and MSW were also attenuated by 26% and 32%, respectively, but remained statistically significant ($P = .003$ and $P < .001$, respectively). Supplementary Figure 1 illustrates differences in CD4/CD8 ratios in both CMV-positive and CMV-negative subgroups. Cytomegalovirus IgG titers were not associated with a lower CD4/CD8 ratio, independently of CMV serostatus, and therefore were not included in the models. Although other factors were independently associated with the CD4/CD8 ratio, they had little to no effect on the low-risk MSM and high-risk MSM coefficients. Table 3 shows the associations of the included covariates on the lymphocyte counts in the final multivariable models from Figure 3. Other than baseline CMV serostatus, recent 3,4-methylenedioxymethamphetamine ([MDMA] $P = .02$) use, a recent diagnosis of syphilis ($P = .01$) or gonorrhea ($P = .005$), and having chronic hepatitis C at baseline ($P = .01$) were also significantly associated with lower CD4/CD8 ratios. A history of tobacco smoking (expressed as pack-years smoking, $P = .01$), a blood draw later in the day (ie, diurnal effect, $P = .009$), and time since baseline ($P < .001$) were associated with a higher CD4/CD8 ratio. Higher I-FABP levels were univariably associated with a lower CD4/CD8 ratio ($P = .009$) but no longer in the multivariable model ($P = .09$). Soluble CD14, sCD163, IL-6, high-sensitivity C-reactive protein (hs-CRP), and D-dimer were not associated with the CD4/CD8 ratio (Supplementary Table 2). Sensitivity analyses limited to cross-sectional data from just the baseline visit showed similar results: after adjustment
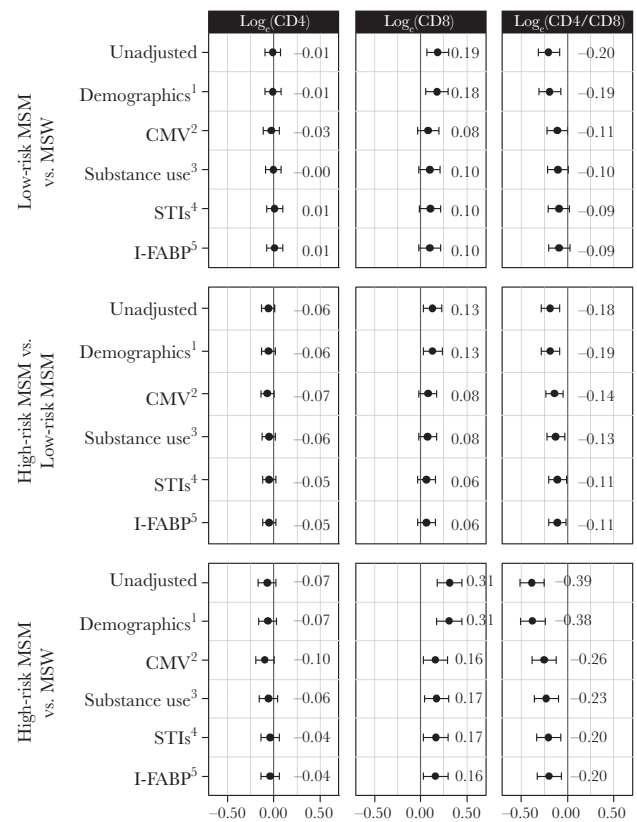
Figure 3. Differences in CD4- and CD8-counts and CD4/CD8 ratios among men who only have sex with women (MSW), low-risk men who have sex with men (MSM), and high-risk MSM, before and after stepwise forward adjustment for additional potential mediating factors. Values are the coefficients from differences between risk groups with 95% confidence intervals extracted from linear mixed-effects models. Adjustment was performed for the following: [1]educational level, time of blood draw, time since baseline; [2]demographics plus cytomegalovirus (CMV) serostatus at baseline; [3]demographics, CMV, plus pack-years smoking, recent (<6 months) 3,4-methylenedioxymethamphetamine (MDMA) use; [4]demographics, CMV, substance use, plus recent (<1–2 years) syphilis or gonorrhea infections, hepatitis C RNA positivity at baseline; [5]demographics, CMV, substance use, sexually transmitted infections (STIs), plus intestinal fatty acid-binding protein (I-FABP) level (ie, marker of microbial translocation). Not independently associated with the CD4/CD8 ratio and therefore not included in the models: age, body mass index, country of origin, current smoking status, recent (<6 months), GHB use, popper use, cocaine use, daily marijuana use, hepatitis B surface antigen positivity at baseline, recent (<1–2 years) chlamydia infection. MSM, men who have sex with men; MSW, men who only have sex with women.

for CMV-serostatus and other covariates, high-risk MSM still had a significantly lower CD4/CD8 ratio ($P = .006$) and higher CD8 count ($P = .06$) compared with MSW. Effect sizes were comparable to those found in the full models [$\log_e$(CD4/CD8) high-risk MSM vs MSW −0.19 (baseline only) and −0.20 (full model); $\log_e$(CD8) high-risk MSM vs MSW 0.13 (baseline only) and 0.16 (full model)].

High CD8 Count and Low CD4/CD8 Ratio Associated With T-Cell Activation and Senescence

In the subgroup of participants with available data, both high CD8 counts and low CD4/CD8 ratios were strongly associated with

Table 3. Associations of Factors Potentially Driving Differences in CD4/CD8 Ratios, CD4 and CD8 Counts, Between High- and Low-Risk MSM and MSW

Variable	FX/TU[a]	Log$_e$ (CD4) All Measurements		Log$_e$ (CD8) All Measurements		Log$_e$ (CD4/CD8) All Measurements	
		Coeff	*P* Value	Coeff	*P* Value	Coeff	*P* Value
Including All Follow-up Measurements							
Risk Category							
MSW	FX	ref	ref	ref	ref	ref	ref
Low-risk MSM	FX	0.01	.8	0.10	.10	−0.09	.12
High-risk MSM	FX	−0.04	.4	**0.16**	**.018**	**−0.20**	**.003**
High educational attainment[b]	FX	−0.01	.8	0.07	.1	−0.08	.05
Time of blood draw (per hour later in the day)	TU	**0.02**	**<.001**	0.01	.1	**0.01**	**.009**
CMV seropositive at baseline	FX	**0.09**	**.02**	**0.41**	**<.001**	**−0.32**	**<.001**
Pack-years smoking per 5 pack-years[c]	TU	**0.03**	**<.001**	**0.01**	**.02**	**0.01**	**.01**
At least monthly use of MDMA during last 6 months	TU	−0.01	.7	0.06	.2	**−0.09**	**.02**
Recent syphilis diagnosis[d]	TU	−0.06	.2	0.05	.4	**−0.12**	**.01**
Recent gonorrhea diagnosis[e]	TU	**−0.07**	**.01**	0.01	.8	**−0.08**	**.005**
Hepatitis C RNA positive at baseline	FX	0.16	.3	**0.68**	**.001**	**−0.52**	**.01**
I-FABP (ng/mL, per log$_e$ increase)	FX	−0.02	.5	0.04	.2	−0.05	.09
Time since baseline in years	TU	−0.00	.6	**−0.02**	**<.001**	**0.02**	**<.001**
Sensitivity Analyses Including Baseline Measurements Only							
Risk Category							
MSW	FX	ref	ref	ref	ref	ref	ref
Low-risk MSM	FX	0.00	.9	0.10	.1	−0.10	.1
High-risk MSM	FX	−0.06	.3	0.13	.06	**−0.19**	**.006**
High educational attainment[b]	FX	0.01	.9	**0.09**	**.04**	**−0.08**	**.05**
Time of blood draw (per hour later in the day)	FX	**0.02**	**.05**	0.00	1.0	0.02	.2
CMV seropositive at baseline	FX	**0.08**	**.04**	**0.43**	**<.001**	**−0.35**	**<.001**
Pack-years smoking per 5 pack-years[c]	FX	**0.03**	**<.001**	0.01	0.05	**0.01**	**.02**
At least monthly use of MDMA during last 6 months	FX	0.02	.8	0.05	.5	−0.03	.7
Recent syphilis diagnosis[d]	FX	−0.06	.6	0.13	.4	−0.19	.2
Recent gonorrhea diagnosis[e]	FX	−0.4	.6	−0.02	.8	−0.02	.9
Hepatitis C RNA positive at baseline	FX	0.23	.2	**0.74**	**.001**	**−0.52**	**.014**
I-FABP (ng/mL, per log$_e$ increase)	FX	−0.02	.4	0.03	.4	−0.05	.1

Abbreviations: Coeff, coefficient; CMV, cytomegalovirus; FX, fixed over time; I-FABP, intestinal-fatty acid binding protein; MSM, men who have sex with men; MSW, men who only have sex with women; MDMA, 3,4-methylenedioxymethamphetamine; ref, reference category; RNA, ribonucleic acid; TU, time updated.

NOTE: Results of multivariable linear mixed-effects models.

[a]Variables are FX or TU.

[b]Higher vocational education or university degree.

[c]One pack-year = 20 cigarettes/day during 1 year.

[d]Rapid plasma reagin >1:4, syphilis diagnosis at sexually transmitted infection (STI) clinic in between visits or <1 year before baseline.

[e]Diagnosis at recruitment visit or in between follow-up visits at the STI clinic.

higher percentages of senescent and activated T-cells, in both the CD4 and CD8 compartments (Supplementary Table 1.2). Moreover, the percentages of activated CD4 and CD8 T cells were significantly higher among high-risk MSM compared with MSW and low-risk MSM (Supplementary Table 1.3). Albeit proportions of senescent CD4 and CD8 T-cells were also numerically higher in high-risk MSM compared with MSW and low-risk MSM, these differences were not statistically significant.

DISCUSSION

In our analysis, middle-aged HIV-negative MSM, especially MSM with a higher number of sex partners, had lower CD4/CD8 ratios, mostly driven by higher CD8 counts, compared with HIV-negative MSW. These differences for a large part were explained by a higher CMV seroprevalence among MSM and high-risk MSM. The remainder of the differences in relative CD4 and CD8 counts could not fully be explained by other factors measured in our study. Several behavioral characteristics and recent STIs were more prevalent among MSM and also independently associated with a lower CD4/CD8 ratio, but they did not influence the low-risk MSM and high-risk MSM coefficients. The independence of these factors (eg, recent syphilis or gonorrhea or MDMA use) should be interpreted with caution. These factors are also strongly correlated with, and therefore a proxy of, high-risk sexual behavior, which might not be fully adjusted for through the other variables in the models. Vieira et al [25] also reported lower CD4 and

higher CD8 counts in pre-exposure prophylaxis-using MSM diagnosed with asymptomatic chlamydia and/or gonorrhea, which was related to increased CD8 T-cell activation, similar to our findings. Furthermore, repeated infection with different CMV strains could be responsible for increased CD8 counts in MSM. Cytomegalovirus-superinfection with a different strain has been demonstrated to occur in humans, most definitively in the context of women already known to be CMV seropositive before becoming pregnant, delivering a child with symptomatic congenital CMV disease [28, 29]. Likewise, in animal models, CMV superinfection was demonstrated and was related to CD8 T-cell expansion [30, 31]. Other viruses, such as herpes simplex virus 1/2, Epstein-Barr virus, and human herpesvirus 8, are also more prevalent among MSM and could contribute to differences in immunological phenotypes [22, 32, 33]. Finally, Noguera-Julian et al [34] showed MSM to have a different gut microbiome compared with MSW irrespective of their HIV status. We found a trend of higher I-FABP (ie, intestinal integrity marker) in those with lower CD4/CD8 ratios. Differences in the composition of the gut microbiome, for example, caused by specific sexual practices or frequent antibiotic use could hypothetically contribute to the lower CD4/CD8 ratios we observed in MSM. Contrary to our findings, previous studies, but conducted only in HIV-positive persons, have reported higher sCD14, sCD163, hs-CRP, and IL-6 levels associated with a lower CD4/CD8 ratio [4, 35]. Specifically, Serrano-Villar et al [4] reported such associations related only to lower CD4 counts, but not higher CD8 counts, and to be absent in HIV-positive participants with >500 CD4 cells/mm^3. In PWH, the association between lower CD4/CD8 ratios and increased innate immune activation markers therefore seems predominantly related to HIV-specific factors, in conjunction with poor CD4 count recovery after cART initiation. The association between a lower CD4/CD8 ratio and higher CD8 T-cell activation in the subgroup of our HIV-negative participants, with higher degrees of T-cell activation in high-risk MSM, resembles findings concerning T-cell activation in HIV-positive individuals [4]. However, our subgroup is insufficiently large and lacks the required follow-up time to demonstrate whether these changes in HIV-negative MSM are associated with increased risk of non-AIDS comorbidity and mortality as was shown in PWH [4].

Strengths and Limitations

The strengths of this study are the relatively large sample of MSM with a sexual risk behavior profile comparable to that of many HIV-positive MSM and the measurement of CD4 and CD8 counts at multiple time points, allowing us to account for the high variability of these indices over time. The extensive data collection including STI-positivity, immune activation markers, and data on sexual behavior and recreational drug use allowed us to explore a multitude of potential factors driving the observed differences in T lymphocyte counts. Important

limitations include that data on chlamydia and gonorrhea infections were not systematically collected, implying that reported diagnosed STIs had been treated at the time of lymphocyte phenotyping, and those asymptomatically present during study visits may have remained undiagnosed. Furthermore, CMV and hepatitis B and C status were only determined at baseline. Thus, incident super- or (re)infections with these viruses during follow-up could not be accounted for. Any such undiagnosed infections could have influenced our results, potentially explaining the remainder of the observed effect of being MSM. However, the cross-sectional baseline-only sensitivity analysis suggested other causative mechanisms, because STI data at baseline were complete. Furthermore, the use of self-reported sexual behavior data may potentially under- or overestimate true exposures, because participants may be inclined to provide socially acceptable answers and be subject to recall bias. The strong correlation of self-reported data with biomarkers of sexual risk such as STI incidence suggests that such bias may be limited in our study. Generalizability of these findings to the general population of MSW and MSM is limited given recruitment of participants at a sexual health clinic, who are more likely to have exhibited more sexual risk behavior. Finally, the classification of low-risk and high-risk behavior based on ≤/>10 sex partners was data-driven and therefore might not be reproducible in other cohorts.

CONCLUSIONS

We observed lower CD4/CD8 ratios, mainly resulting from higher CD8 counts, in HIV-negative MSM compared with MSW. We show that these differences could at least partially be explained by higher CMV seroprevalence in MSM. Men who have sex with men have high rates of STIs, but they also have psychosocial stress due to a social minority position [36], often in conjunction with higher rates of recreational drug use. Future studies should attempt to evaluate the possible influence of such factors on immune system changes. In turn, these changes may be related to increased cardiovascular disease risk observed in both HIV-positive and HIV-negative MSM [37, 38]. For example, CMV seroprevalence was much higher among MSM in our study and has been associated with cardiovascular disease development, frailty, and mortality [39–46]. Our findings contribute to the understanding of why in many HIV-positive MSM who are treated with cART and have suppressed viremia, the CD4/CD8 ratio and CD8 counts do not return to general population-based normal values. Many PWH differ in their lifestyle compared with people from the general population. We show that such factors or exposures can impact their immune system. These immunological differences should not mistakenly be attributed only to HIV infection or its treatment in studies using general population control subjects. Our results also warrant further investigation into the effects of (CMV-induced) immunological differences on the occurrence

of age-related comorbidities not only in HIV-positive but also HIV-negative MSM.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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