



BST-2 Expression Modulates Small CD4-Mimetic Sensitization of HIV-1-Infected Cells to Antibody-Dependent Cellular Cytotoxicity

Jonathan Richard,^{a,b} J  r  mie Pr  vost,^{a,b} Benjamin von Bredow,^c Shilei Ding,^{a,b} Nathalie Brassard,^a Halima Medjahed,^a Mathieu Coutu,^a Bruno Melillo,^d Fr  d  ric Bibollet-Ruche,^e Beatrice H. Hahn,^e Daniel E. Kaufmann,^{a,f,g} Amos B. Smith, III,^d Joseph Sodroski,^{h,i} Daniel Sauter,^j Frank Kirchhoff,^j Katrina Gee,^k Stuart J. Neil,^l David T. Evans,^{c,m} Andr  s Finzi^{a,b,n}

Centre de Recherche du CHUM, Montreal, Quebec, Canada^a; Department of Microbiology, Infectiology and Immunology, Universit   de Montr  al, Montreal, Quebec, Canada^b; Department of Pathology and Laboratory Medicine, University of Wisconsin—Madison, Madison, Wisconsin, USA^c; Department of Chemistry, School of Arts and Sciences, University of Pennsylvania, Philadelphia, Pennsylvania, USA^d; Departments of Medicine and Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA^e; Department of Medicine, Universit   de Montr  al, Montreal, Quebec, Canada^f; Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, The Scripps Research Institute, La Jolla, California, USA^g; Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, and Department of Microbiology and Immunobiology, Division of AIDS, Harvard Medical School, Boston, Massachusetts, USA^h; Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts, USAⁱ; Institute of Molecular Virology, Ulm University Medical Center, Ulm, Germany^j; Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada^k; Department of Infectious Disease, King's College London School of Life Sciences and Medicine, Guy's Hospital, London, United Kingdom^l; Wisconsin National Primate Research Center, University of Wisconsin—Madison, Madison, Wisconsin, USA^m; Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canadaⁿ

ABSTRACT Antibodies recognizing conserved CD4-induced (CD4i) epitopes on human immunodeficiency virus type 1 (HIV-1) Env and able to mediate antibody-dependent cellular cytotoxicity (ADCC) have been shown to be present in sera from most HIV-1-infected individuals. These antibodies preferentially recognize Env in its CD4-bound conformation. CD4 downregulation by Nef and Vpu dramatically reduces exposure of CD4i HIV-1 Env epitopes and therefore reduce the susceptibility of HIV-1-infected cells to ADCC mediated by HIV-positive (HIV+) sera. Importantly, this mechanism of immune evasion can be circumvented with small-molecule CD4 mimetics (CD4mc) that are able to transition Env into the CD4-bound conformation and sensitize HIV-1-infected cells to ADCC mediated by HIV+ sera. However, HIV-1 developed additional mechanisms to avoid ADCC, including Vpu-mediated BST-2 antagonism, which decreases the overall amount of Env present at the cell surface. Accordingly, BST-2 upregulation in response to alpha interferon (IFN- α) was shown to increase the susceptibility of HIV-1-infected cells to ADCC despite the activity of Vpu. Here we show that BST-2 upregulation by IFN- β and interleukin-27 (IL-27) also increases the surface expression of Env and thus boosts the ability of CD4mc to sensitize HIV-1-infected cells to ADCC by sera from HIV-1-infected individuals.

IMPORTANCE HIV-1 evolved sophisticated strategies to conceal Env epitopes from ADCC-mediating antibodies present in HIV+ sera. Vpu-mediated BST-2 downregulation was shown to decrease ADCC responses by limiting the amount of Env present at the cell surface. This effect of Vpu was shown to be attenuated by IFN- α treatment. Here we show that in addition to IFN- α , IFN- β and IL-27 also affect Vpu-mediated BST-2 downregulation and greatly enhance ADCC responses against HIV-1-

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Address correspondence to Jonathan Richard, jonathan.richard.1@umontreal.ca, or Andr  s Finzi, andres.finzi@umontreal.ca.

infected cells in the presence of CD4mc. These findings may inform strategies aimed at HIV prevention and eradication.

KEYWORDS HIV-1, BST-2, envelope glycoproteins, gp120, CD4, CD4-bound conformation, nonneutralizing antibodies, ADCC, CD4 mimetics, IFN- α , Env, IL-27, interferons

Antibodies that preferentially recognize the CD4-bound conformation of human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins (Env) can eliminate HIV-1-infected cells through antibody-dependent cellular cytotoxicity (ADCC) responses (1–4). These antibodies are present in serum (1, 5), breast milk (5), and cervicovaginal lavage fluid (2, 5) samples from HIV-1-infected individuals and have been proposed to be part of the pressure exerted on HIV-1 to efficiently downregulate CD4 from the cell surface (6). Accordingly, Nef- and Vpu-mediated CD4 downregulation conceal the exposure of Env epitopes recognized by these antibodies (1, 3, 7). In addition, HIV-1 decreases ADCC responses by diminishing the overall amount of Env present at the cell surface. This is achieved through Vpu-mediated BST-2 (tetherin/CD317/HM1.24) downregulation (7–9), which allows for efficient release of viral particles (10, 11), and also through efficient Env internalization mediated by an endocytosis motif in the cytoplasmic tail of gp41 (12).

A better understanding of the importance that the CD4-bound conformation of HIV-1 envelope glycoproteins has on ADCC responses prompted us to “force” this Env conformation on the surface of infected cells using small-molecule CD4 mimetics (CD4mc). CD4mc induction of the CD4-bound conformation results in enhanced recognition of HIV-1-infected cells by serum, breast milk, and cervicovaginal fluid samples from HIV-1-infected subjects. Most importantly, CD4mc sensitizes HIV-1-infected cells to ADCC responses mediated by these biological fluids (4, 5, 13).

The effect of CD4mc on ADCC responses may be influenced by the amount of Env available at the cell surface. Only limited amounts of Env are presented at the cell surface due to efficient Env internalization (12) and Vpu-mediated BST-2 downregulation (7–9); this places an upper limit on the amount of Env that can be rendered susceptible to ADCC by CD4mc. Interestingly, two BST-2 isoforms possessing distinct biological properties have been described (14, 15). While the long isoform of BST-2 (L-BST-2) contains a cytoplasmic tyrosine motif mediating endocytic recycling, sensitivity to HIV-1 M Vpu and innate immune sensing, the short isoform of BST-2 (S-BST-2) lacks this motif due to the utilization of an alternative start codon (14, 15). How these two isoforms modulate Env recognition on the surface of HIV-1-infected cells by HIV-positive (HIV+) sera and how this affects the activity of CD4mc remain unknown.

Type I interferons (IFNs) are an important part of the early host immune response observed during acute HIV-1 infection (16). The antiviral effect exerted by IFN is highlighted by the observation that transmitted/founder HIV-1 strains that initiate host infection have been shown to be more resistant to type I IFN responses than HIV-1 strains found during the chronic phase of infection (17–19). Furthermore, Vpu enhances viral replication particularly during early stages of infection, probably by counteracting the IFN-inducible restriction factor BST-2 (18, 20). The induction of BST-2 expression by type I IFN treatment was also shown to sensitize infected cells to ADCC (8). In addition, interleukin-27 (IL-27) also enhances BST-2 levels on the surface of human monocytes and CD4 T cells (21). IL-27 is a member of the IL-12 family of cytokines and drives the differentiation of Th1 CD4 T cells (22, 23). Interestingly, IL-27 induces an antiviral gene expression profile similar to that induced by alpha interferon (IFN- α), including the *apobec3g* gene (24). Furthermore, IL-27 inhibited the replication of HIV-1 in cultures of primary CD4⁺ T cells and monocytes/macrophages through the induction of APOBEC (apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like) proteins (24, 25). Notably, IL-27-mediated BST-2 upregulation was shown to be independent from type I IFN responses (21). However, the effect of IL-27 on ADCC responses during viral infection has not been determined.

Here we evaluated the role of BST-2 on Env accumulation on the surface of HIV-1-infected cells and tested whether type I IFNs or IL-27 could be exploited in conjunction with CD4mc to further enhance ADCC responses mediated by HIV-positive (HIV+) sera.

RESULTS

BST-2 expression modulates Env accumulation on the surface of HIV-1-infected cells and its recognition by HIV+ sera in the presence of CD4mc. In the absence of Vpu, Env accumulates at the plasma membrane of HIV-1-infected cells (7–9) in large part due to the inhibitory effects of BST-2 on virus release (10, 11). This surface accumulation results in increased susceptibility of HIV-1-infected cells to ADCC (7–9). To further evaluate the role of BST-2 on Env surface expression, we infected Jurkat cell lines expressing no BST-2 (Jurkat Tag) or expressing the long isoform of BST-2 (Jurkat Tag L-BST-2) or the short isoform of BST-2 (Jurkat Tag S-BST-2) (15). Cells were infected with the transmitted/founder virus CH58 (CH58 TF) (5) expressing the Vpu accessory protein (wild-type [wt] CH58 TF) or containing a *vpu* deletion (Vpu⁻). Forty-eight hours postinfection, BST-2 and Env levels were evaluated by cell surface staining followed by intracellular p24 staining to identify infected (p24-positive [p24+]) cells. As expected, while BST-2 was not detected on the surface of Jurkat Tag cells (Fig. 1A and D), it was equivalently detected on the surface of uninfected (mock) Jurkat Tag L-BST-2 and S-BST-2 cells, indicating that these two cell lines express similar levels of BST-2 (Fig. 1B to D). However, in agreement with previous reports, HIV-1 infection significantly decreased expression of L-BST-2 but not that of S-BST-2. The S-BST-2 isoform lacks 12 residues of the cytoplasmic tail required for Vpu group M-mediated BST-2 endosomal degradation (14, 15) (Fig. 1C and D). As expected, a virus lacking Vpu (Vpu⁻) was unable to decrease cell surface levels of BST-2 (Fig. 1B to D).

When we evaluated Env levels on the surface of infected cells with the conformation-independent 2G12 antibody (Fig. 2A), we observed a significant correlation with BST-2 levels (Fig. 2B). This supports previous observations indicating that BST-2 modulates the overall amount of Env on the surfaces of infected cells (7, 8). We then assessed whether enhanced accumulation of Env affected recognition of HIV-1-infected cells by HIV+ sera. Despite different amounts of BST-2 and Env present on the surface of Jurkat cell lines expressing S-BST-2, L-BST-2, or no BST-2, cells infected with a wild-type virus were barely recognized by HIV+ sera (Fig. 2C). This is believed to reflect the ability of HIV-1 to downregulate CD4 in infected cells such that only closed Env trimers remain (1, 3, 5, 7, 26). Antibodies present in HIV+ sera preferentially recognize Env in its CD4-bound conformation (1, 27). Infection with a *vpu*⁻ virus led to a small increase in recognition of HIV-1-infected cells by HIV+ sera (Fig. 2C). In order to expose epitopes recognized by antibodies present in HIV+ sera, infected cells were incubated in parallel with the potent CD4mc BNM-III-170 which forces Env to adopt a CD4-bound-like conformation (4, 28) and in conjunction with coreceptor binding site antibodies (CoRBS) efficiently expose anti-cluster A epitopes (4). CD4mc addition enhanced recognition of all three infected cell lines (Fig. 2C). In agreement with decreased sensitivity of S-BST-2 to Vpu-mediated downregulation, Jurkat Tag S-BST-2 cells infected with a wild-type virus responded significantly better to CD4mc than Jurkat Tag L-BST-2 cells infected with the same virus. This likely results from an enhanced Env accumulation on the surfaces of Jurkat Tag S-BST-2 cells due to the inability of Vpu to downregulate S-BST-2 (Fig. 2A and C). Likely due to the absence of BST-2 in the Jurkat Tag empty vector (EV) cell line, infection with a *vpu*⁻ virus has a minor effect on Env levels (as evaluated by 2G12) and therefore recognition of infected cells by HIV+ sera in the absence of CD4mc. Infection with a Vpu⁻ virus of Jurkat Tag S-BST-2 and L cells led to a slightly better recognition of infected cells in the absence of CD4mc. Upon addition of CD4mc, however, all infected cells were significantly better recognized by HIV+ sera. This finding confirms previous observations indicating that antibodies present in HIV+ sera preferentially recognize Env in the CD4-bound conformation (1). Of note, the difference in HIV+ serum recognition in the presence of CD4mc between wild-type and *vpu*⁻ virus-

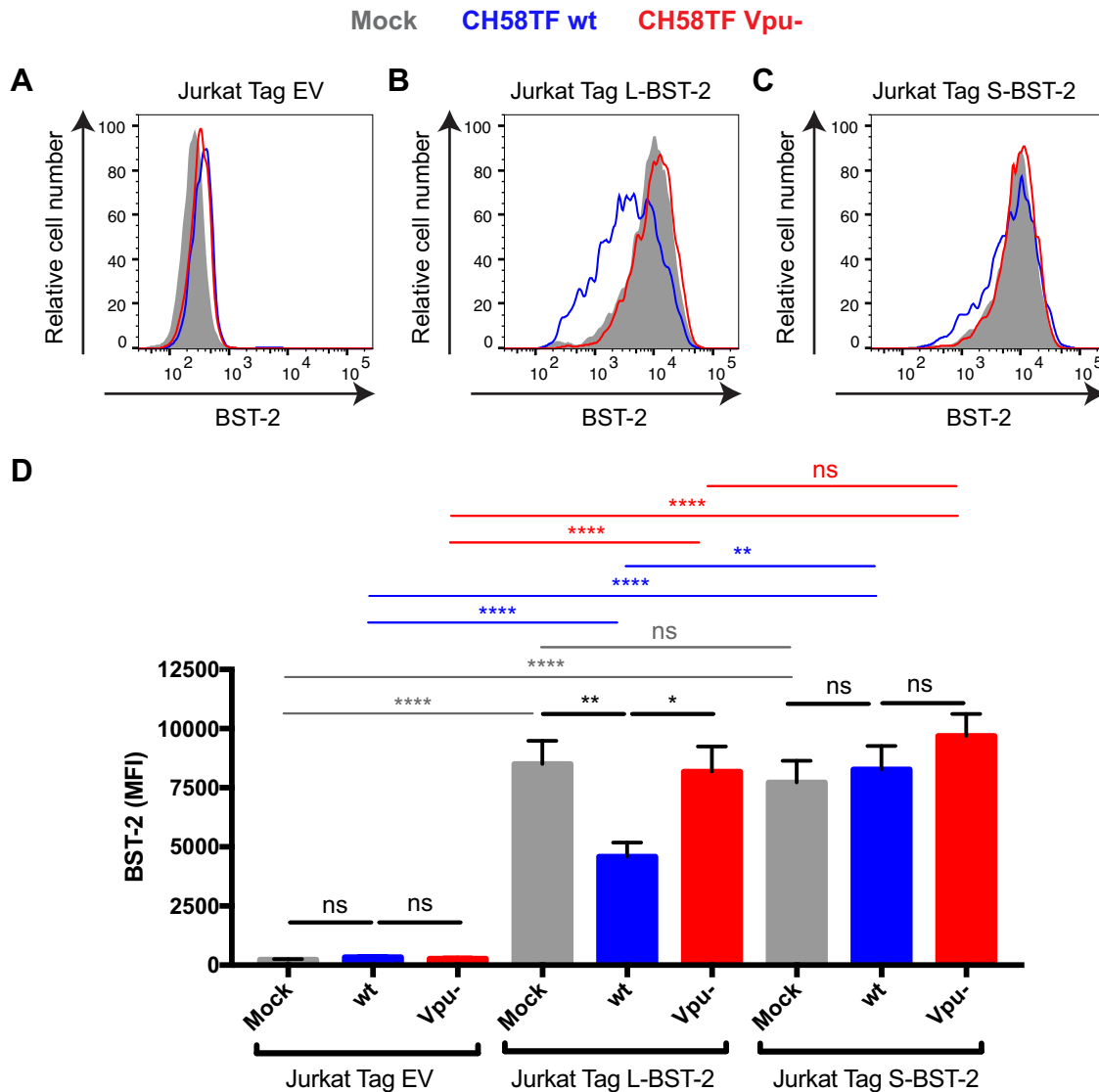


FIG 1 Differential sensitivity of BST-2 isoforms to HIV-1 Vpu in Jurkat cell lines. Jurkat Tag cells (A and D) expressing no BST-2 (Jurkat Tag EV [empty vector]) or stably expressing the L-BST2 (B and D) or S-BST-2 (C and D) were mock infected or infected with the transmitted/founder virus HIV-1 CH58 (CH58TF) expressing Vpu (wild-type CH58TF [CH58TF wt]) or not expressing Vpu (CH58TF Vpu-). Forty-eight hours postinfection, cells were stained with anti-BST-2 Ab, followed with appropriate secondary Abs. (A to C) Histograms depicting representative staining; (D) mean fluorescence intensity (MFI) obtained in at least six independent experiments. Values are means plus standard error of the means (SEM) (error bars). Statistical significance was tested using an unpaired *t* test (*, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$; ns, nonsignificant).

infected cells was higher in Jurkat L-BST-2 cells that express the BST-2 isoform susceptible to Vpu action (Fig. 2C). Accumulation of Env (as measured by 2G12) correlated significantly with recognition of infected cells by HIV+ sera (Fig. 2D). Recognition of infected cells by HIV+ sera also correlated with BST-2 expression (Fig. 2E).

BST-2 levels regulate Env accumulation and its recognition by HIV+ sera on the surface of HIV-1-infected primary CD4⁺ T cells. Figures 1 and 2 showed that BST-2 levels, and its sensitivity to Vpu downregulation, dictated Env accumulation on the surfaces of HIV-1-infected cell lines. Moreover, Env accumulation on the surfaces of infected cells increased the amount of Env available to engage CD4mc and henceforth sample the CD4-bound conformation, which is preferentially recognized by HIV+ sera (1, 27). IFN- α treatment has been shown to enhance BST-2 levels, resulting in an accumulation of Env on the surfaces of HIV-1-infected cells and thus increasing the sensitivity of HIV-1-infected cells to ADCC (8). Similar observations were recently

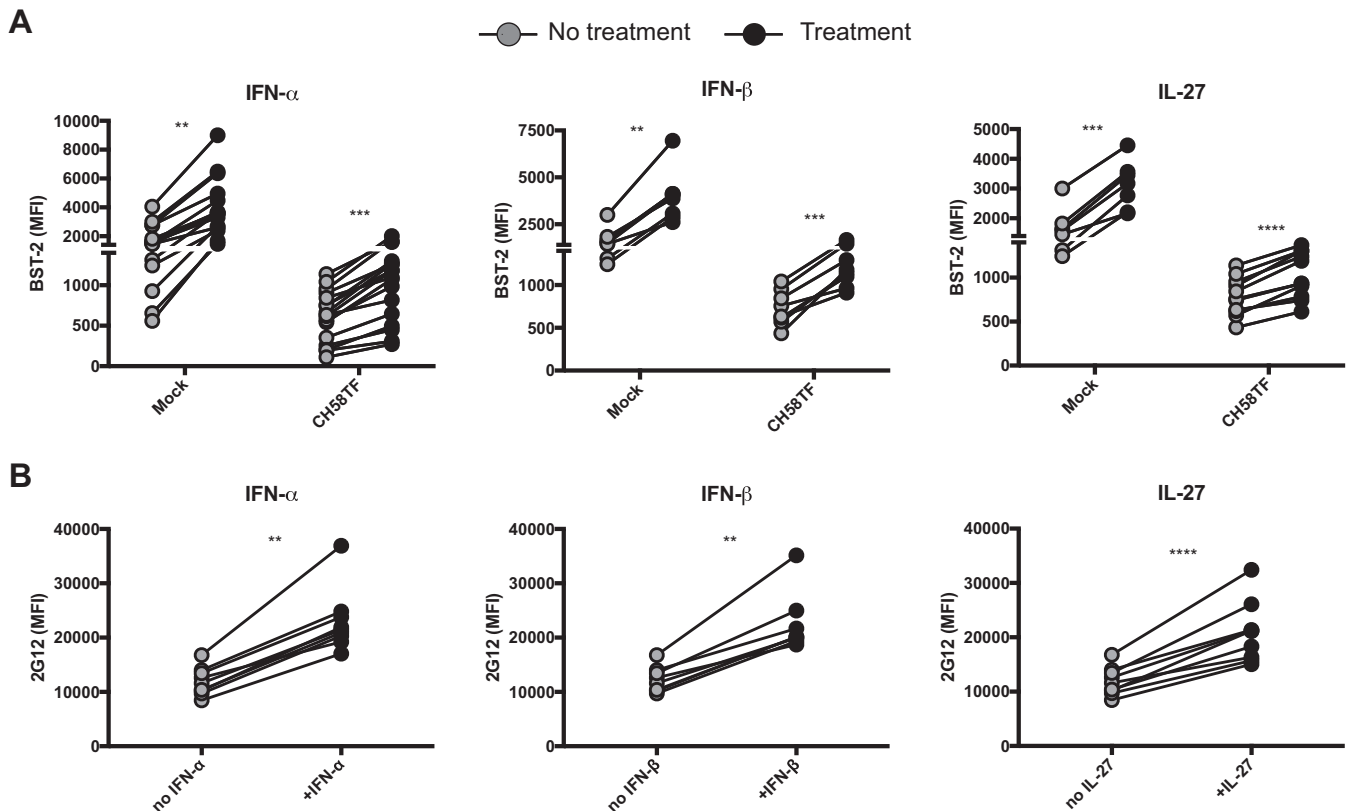


FIG 3 Treatment with type I IFN or IL-27 enhances Env levels on the surface of HIV-1-infected cells through BST-2 upregulation. Primary CD4⁺ T cells were mock infected or infected with the transmitted/founder virus CH58 (CH58TF) and either treated for 24 h with type I IFN (IFN- α and IFN- β) or IL-27 or not treated. Forty-eight hours postinfection, cells were stained with anti-BST-2 Ab (A) or anti-Env Ab 2G12 (B), followed with appropriate secondary Abs. The graphs shown represent the mean fluorescence intensities obtained for at least eight independent experiments. Statistical significance was tested using a paired *t* test or Wilcoxon matched-pair signed-rank test based on statistical normality (**, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

reported (29). Therefore, we decided to take advantage of the type 1 interferon responsiveness of BST-2 (10, 11). Primary CD4⁺ T cells from healthy HIV-1 uninfected individuals were mock infected or infected with HIV-1 (CH58 TF), and BST-2 levels were modulated by stimulation with type 1 IFNs (IFN- α and IFN- β) or with IL-27 (21). As expected, BST-2 levels were significantly higher in uninfected than HIV-1-infected cells. Interestingly, IFN- α , IFN- β , and IL-27 treatment enhanced BST-2 detection in both uninfected and HIV-1-infected cells (Fig. 3A). BST-2 upregulation resulted in Env accumulation on the surfaces of infected cells, as measured with the 2G12 antibody (Fig. 3B).

We then evaluated whether IFN- α , IFN- β , and IL-27 treatment enhanced recognition of HIV-1-infected cells by HIV+ sera. Despite a significant increase in Env accumulation on the surfaces of infected cells (Fig. 3B), treatment with IFN- β and IL-27 failed to enhance recognition of infected cells by HIV+ sera, and the effect of IFN- α treatment was relatively minor (Fig. 4). However, addition of the CD4mc BNM-III-170 significantly increased recognition of HIV-1-infected cells by HIV+ sera; these results are in agreement with previous reports demonstrating the ability of HIV+ sera to recognize CD4i epitopes on primary HIV-1 Env that are not spontaneously exposed (1, 3) and the capacity of CD4mc to promote the CD4-bound conformation of Env on the surfaces of HIV-1-infected cells (4, 5, 13, 30). Remarkably, the combination of IFN- α , IFN- β , or IL-27 with BNM-III-170 further increased recognition of HIV-1-infected cells by all sera tested compared to any one of these treatments (Fig. 4).

BST-2 upregulation boosts the capacity of CD4mc to sensitize HIV-1-infected cells to ADCC mediated by HIV+ sera. To evaluate whether the enhanced recognition of HIV-1-infected cells induced by the combination of IFN- α , IFN- β , and IL-27 treatments and BNM-III-170 would result in enhanced ADCC killing, we infected primary

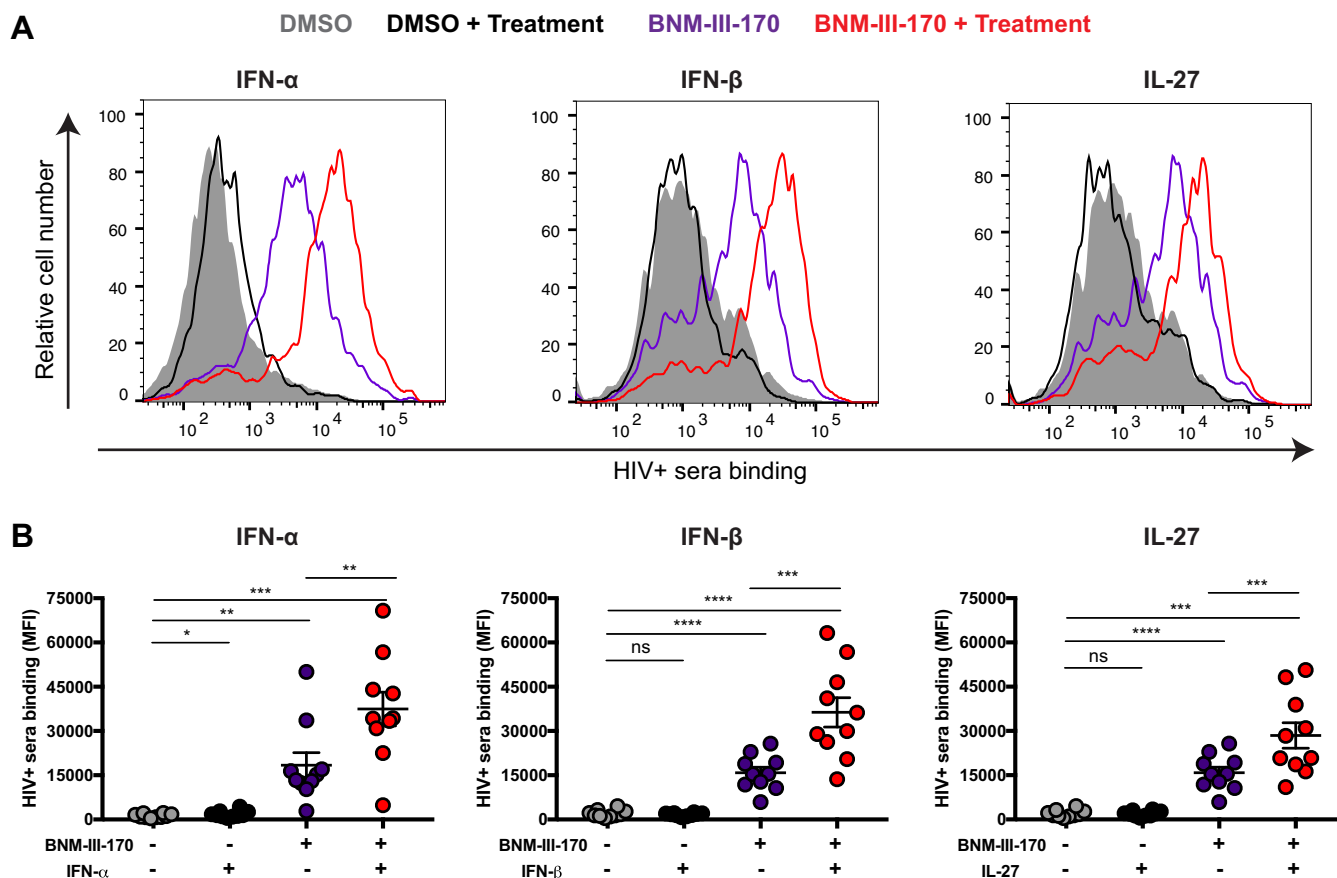


FIG 4 Treatment with type I IFN or IL-27 enhances recognition of HIV-1-infected cells by sera from HIV-1-infected individuals in the presence of CD4mc. Primary CD4⁺ T cells were mock infected or infected with the transmitted/founder virus HIV-1 CH58 (CH58 T/F) and either treated for 24 h with type I IFN (IFN- α and IFN- β) or IL-27 or not treated. Forty-eight hours postinfection, cells were stained with sera from 10 HIV-1-infected individuals in the presence of the CD4mc BNM-III-170 (50 μ M) or an equivalent volume of DMSO, followed with appropriate secondary Abs. (A) Histograms depicting representative staining; (B) mean fluorescence intensities (MFI) obtained with all the different sera. Values are means \pm standard error of the means (SEM) (error bars). Statistical significance was tested using a paired *t* test or Wilcoxon matched-pair signed-rank test based on statistical normality (*, *P* < 0.05; **, *P* < 0.01, ***, *P* < 0.001; ****, *P* < 0.0001; ns, nonsignificant).

CD4⁺ T cells with HIV-1 CH58 TF and evaluated their susceptibility to ADCC mediated by autologous peripheral blood mononuclear cells (PBMCs) using a previously described fluorescence-activated cell sorting (FACS)-based assay (5, 31). As reported (5, 13), CD4mc BNM-III-170 significantly increased ADCC mediated by all HIV+ sera tested (Fig. 5). In agreement with the recognition of infected cells by HIV+ sera (Fig. 4), IFN- α treatment alone had a minor but significant effect on ADCC responses (Fig. 5), but IFN- β and IL-27 treatment failed to do so (Fig. 5). Remarkably, addition of BNM-III-170 further enhanced the susceptibility of infected cells to ADCC for cells treated with IFN- α , IFN- β , or IL-27 (Fig. 5). As expected, enhanced recognition of HIV-1-infected cells by HIV+ sera positively correlated with enhanced ADCC responses (Fig. 6). These results highlight the potential of combining type I IFNs and IL-27 with CD4mc to sensitize HIV-1-infected cells to ADCC.

DISCUSSION

Increasing evidence suggests that Fc γ receptor-dependent functions of antibodies play a role in controlling human immunodeficiency virus type 1 (HIV-1) infection and replication (32–40). Analysis of the correlates of protection in the RV144 vaccine trial suggested that decreased HIV-1 acquisition was linked to increased ADCC activity in protected vaccinees (41). ADCC-mediating antibodies (Abs) targeting anti-cluster A epitopes were isolated from some RV144 vaccinees (42) and were shown to preferentially recognize the HIV-1 envelope glycoproteins sampling the CD4-bound conforma-

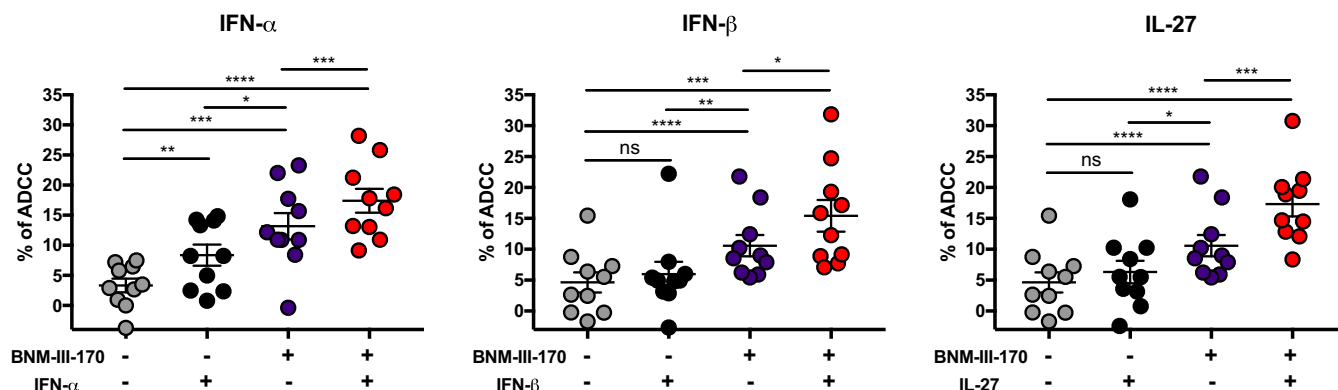


FIG 5 Treatment with type I IFN or IL-27 boosts CD4mc sensitization of HIV-1-infected cells to ADCC. Primary CD4⁺ T cells infected with the transmitted/founder virus CH58 (CH58 T/F), either treated for 24 h with type I IFN (IFN- α and IFN- β) or IL-27 or not treated, were used as target cells, and autologous PBMCs were used as effector cells in our FACS-based ADCC assay. Shown are the percentages of ADCC-mediated killing obtained with sera from 10 HIV-1-infected individuals in the presence of the CD4mc BNM-III-170 (50 μ M) or an equivalent volume of DMSO. Values are means \pm standard error of the means (SEM) (error bars). Statistical significance was tested using a paired *t* test or Wilcoxon matched-pair signed-rank test based on statistical normality (*, *P* < 0.05; **, *P* < 0.01, ***, *P* < 0.001; ****, *P* < 0.0001; ns, nonsignificant).

tion (7). CD4i antibodies represent a significant portion of the anti-Env Abs elicited during natural HIV-1 infection (1, 27, 43). This elicitation of CD4i Abs could result from transitional exposure of CD4i Env epitope during viral entry (44) or, most likely, after binding of shed gp120 with CD4 on uninfected bystander cells (30). However, not all CD4i antibodies are able to mediate ADCC against HIV-1-infected cells. While anti-cluster A antibodies have been shown to mediate potent ADCC responses against infected cells exposing Env in the CD4-bound conformation (3, 4, 7, 45), CD4i antibodies targeting the coreceptor binding site appear to be unable to do so (3, 4, 45, 46). While the reasons for these differences are not fully understood, the angle of approach of the antibody toward Env might differentially expose the Fc region which must be engaged by the Fc γ receptor in order to activate effector cells. Nevertheless, to limit the exposure of anti-cluster A epitopes that are exposed in the CD4-bound conformation of Env on the surfaces of infected cells, HIV-1 evolved sophisticated mechanisms to efficiently internalize Env (12) to counteract the host restriction factor BST-2 with the viral Vpu protein (7–9) and to downregulate CD4 by Nef and Vpu (1, 7). The requirement to evade ADCC provides one plausible explanation of why the vast majority of circulating HIV-1 strains worldwide express functional Nef and Vpu proteins, which limit the exposure of CD4i Env epitopes on the surfaces of infected cells.

In agreement with the necessity for HIV-1 to avoid exposing the CD4-bound conformation of Env, we recently showed that forcing Env to adopt this conformation with CD4mc sensitizes HIV-1-infected cells to ADCC by sera from HIV-1-infected sub-

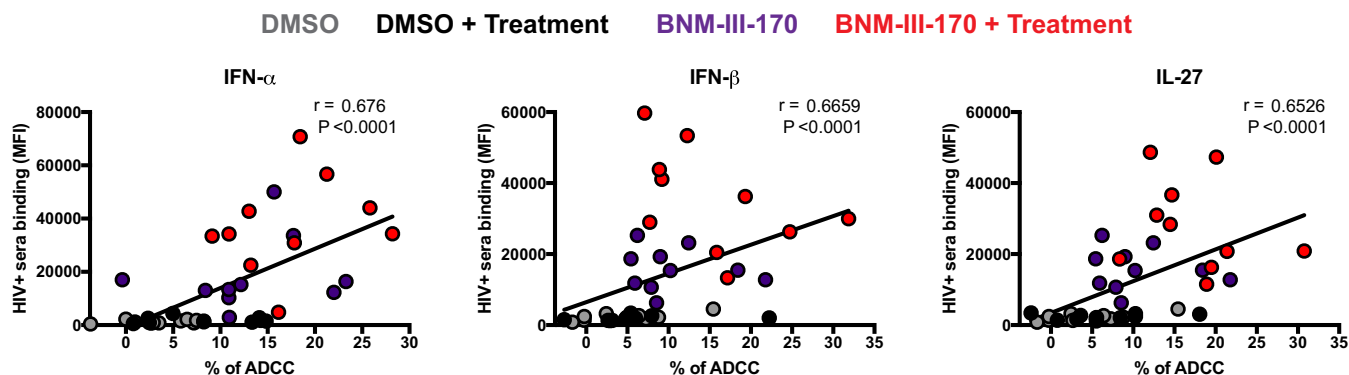


FIG 6 Enhanced recognition of HIV-1-infected cells positively correlates with enhanced ADCC responses. A positive correlation was observed between the recognition of primary CD4 T cells infected with the transmitted/founder virus CH58 (CH58 T/F) by HIV+ sera and their ability to mediate an ADCC response. The correlations obtained for the different treatments (IFN- α , IFN- β , or IL-27) are shown. Statistical analysis was tested utilizing a Spearman rank correlation.

Protection from ADCC

ADCC response

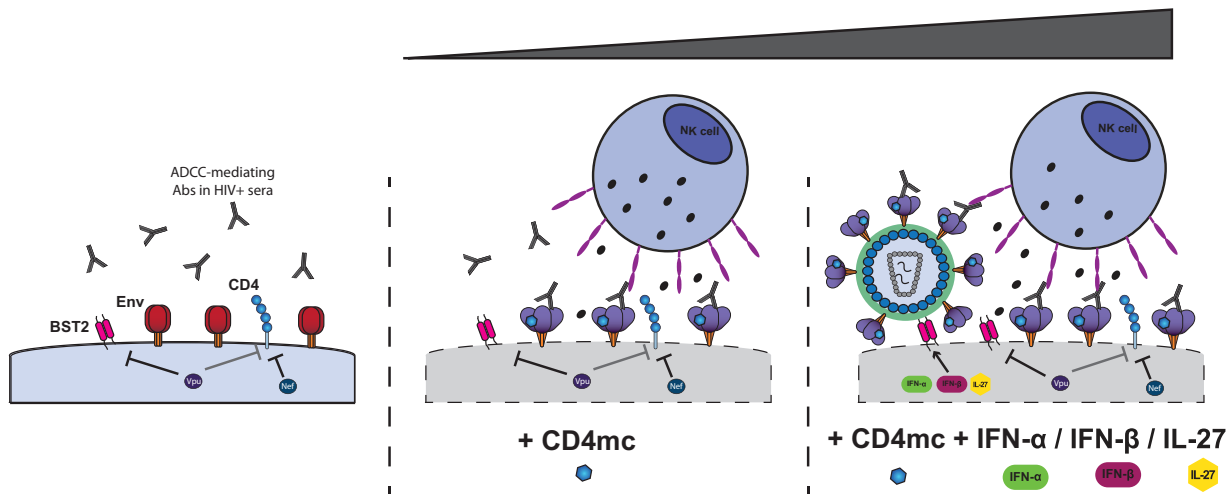


FIG 7 Env conformation and its accumulation at the cell surface dictates sensitivity of HIV-1-infected cells to ADCC. ADCC-mediating Abs present in sera from HIV-1-infected individuals preferentially recognize Env in its CD4-bound conformation (1). To limit the exposure of this conformation, HIV-1 has evolved sophisticated mechanisms to counteract the host restriction factor BST-2 with the viral Vpu protein (7–9) and to downregulate CD4 by Nef and Vpu (7). Nef and Vpu decrease the accumulation of Env and its interaction with CD4 at the cell surface, two factors that determine the susceptibility of HIV-1-infected cells to ADCC. Small CD4 mimetics sensitize HIV-1-infected cells to ADCC mediated by HIV+ sera by forcing Env to sample its CD4-bound conformation (5). Type I IFN or IL-27 treatment, through upregulation of BST-2 despite Vpu activity, boosts the ability of CD4mc by increasing the amounts of CD4mc-sensitized Env available on the cell surface.

jects (5). Here we show that increasing the amounts on Env at the cell surface, once this Env is induced by CD4mc to adopt the CD4-bound conformation, results in increased recognition of HIV-1-infected cells by HIV+ sera. We found that enhanced recognition of infected cells by HIV+ sera translates into enhanced susceptibility of infected cells to ADCC. This was achieved by exploiting the type 1 interferon responsiveness of the restriction factor BST-2, known to trap mature viral particles on the surfaces of infected cells. IFN- α and - β enhance BST-2 levels on the surfaces of infected cells, which translates into enhanced levels of Env potentially able to be targeted by ADCC after engaging the CD4mc. Interestingly, similar results were obtained using IL-27, a cytokine known to modulate BST-2 levels in an IFN-independent manner. Altogether, our results suggest a model (Fig. 7) where the conformation and availability of Env at the cell surface dictates the sensitivity of HIV-1-infected cells to ADCC. HIV-1 limits the amount of Env present at the cell surface and tightly controls its conformation. By preventing Env from assuming the CD4-bound conformation, HIV-1 avoids Env recognition by CD4i ADCC-mediating Abs present in the sera of the majority of HIV-1-infected individuals. Small CD4mc sensitize HIV-1-infected cells to ADCC by forcing Env to expose CD4i anti-cluster A-mediating epitopes. IFN- α , IFN- β , and IL-27 treatment, through upregulation of BST-2, increases the total amounts of Env available for CD4mc to induce ADCC-susceptible Env conformations on the surfaces of infected cells. While HIV-1-infected cells are protected from ADCC responses, we recently demonstrated that uninfected bystander CD4⁺ T cells bind gp120 shed from productively infected cells and are efficiently recognized by ADCC-mediating antibodies (30). Importantly, we also demonstrated that this phenomenon can be blocked by CD4mc that abrogates the binding of gp120 to uninfected cells and effectively redirects the immune system to infected cells. Therefore, the combination of CD4mc and type I IFN or IL-27 would represent an effective strategy to specifically target and eliminate HIV-1-infected cells by ADCC.

Robust type I interferon responses are among the earliest host immune defenses observed during acute HIV-1 infection (16). Accordingly, transmitted/founder viruses, including those used in the present study, were found to be more resistant to IFN treatment than viruses from chronic HIV-1 infection (17–19). In that context, Vpu

counteraction of BST-2 was recently identified as a major determinant of this IFN resistance (18, 20) and was found to play a crucial role in enhancing virus replication and release in human CD4⁺ T cells, particularly in the presence of IFN (18). Here we found that IFN- α , IFN- β , or IL-27 treatment enhanced BST-2 levels and, in combination with CD4mc, similarly sensitized HIV-1-infected cells to ADCC. However, there are many other IFN- α subtypes, and some of them inhibit HIV-1 replication more efficiently *in vitro* and in animal models than IFN- α 2 (47, 48). Thus, it will be important to evaluate to what extent the different IFN- α subtypes sensitize HIV-1-infected cells to ADCC in the presence of CD4mc.

CD4mc were recently shown to enhance the viral neutralization and ADCC activities of antibodies elicited in nonhuman primates (NHP) by several different Env immunogens (49), suggesting that combining a vaccine with a small-molecule CD4mc, administered orally or in a microbicide formulation, might be useful as a prophylactic strategy against HIV-1 transmission. Interestingly, mucosal application of IFN- β protected macaques from intrarectal and intravaginal simian-human immunodeficiency virus (SHIV) challenges (50). Similarly, IFN- α 2 treatment of rhesus macaques prevented systemic infection by simian immunodeficiency virus (SIV) (51). Whereas a combination of IFNs or IL-27 with CD4mc might further limit HIV-1 transmission or help decrease the size of the viral reservoir in HIV-1-infected individuals remains to be evaluated, our results support performing future experiments aimed at evaluating whether sensitization of HIV-infected cells to ADCC could affect viral transmission and/or replication in animal models.

MATERIALS AND METHODS

Cell lines and isolation of primary cells. HEK293T human embryonic kidney (obtained from ATCC) and primary cells were grown as previously described (7, 52). Peripheral blood mononuclear cells (PBMCs) were obtained by leukapheresis. All participants provided informed written consent prior to enrollment in accordance with Institutional Review Board approval. CD4 T lymphocytes were purified from resting PBMCs by negative selection and activated as previously described (5). Jurkat Tag cells stably expressing the long isoform of BST-2 (L-BST-2) or the short isoform of BST-2 (S-BST-2) and the Jurkat Tag empty vector (EV) cell line expressing no BST-2 were previously described (15).

Viral production, infections, and detection of infected cells. In order to achieve the same level of infection between wild-type (wt) and Vpu⁻ viruses, vesicular stomatitis virus G (VSVG)-pseudotyped HIV-1 replicating competent viruses were produced. Briefly, proviral vectors and a VSVG-encoding plasmid were cotransfected in 293T cells by standard calcium phosphate transfection. Two days after transfection, cell supernatants were harvested, clarified by low-speed centrifugation (5 min at 1,500 rpm), and concentrated by ultracentrifugation for 1 h at 4°C at 100,605 \times g over a 20% sucrose cushion. Pellets were resuspended in fresh RPMI 1640 medium, and aliquots were stored at -80°C until use (1). Viruses were then used to infect Jurkat Tag cell lines or primary CD4 T cells from healthy donors by spin infection at 800 \times g for 1 h in 96-well plates at 25°C.

CD4 mimetic, type I IFN, or IL-27 treatments. The CD4mc BNM-III-170 was synthesized as described previously (28). BNM-III-170 was dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 10 mM, aliquoted, and stored at -20°C. BNM-III-170 was then diluted to 50 μ M in phosphate-buffered saline (PBS) for cell surface staining or in complete RPMI 1640 medium for ADCC assays. IFN- α (PBL Assay Science) was reconstituted in complete RPMI 1640 medium at 1 \times 10⁷ U/ml, aliquoted, and stored at -80°C. IFN- α was then added to the cells at 1,000 U/ml. IFN- β (Rebif; EMD Serono Inc.) (19) was added to the cells at 1 ng/ml. IL-27 (R&D Systems) was reconstituted at 100 μ g/ml in sterile PBS containing 0.1% bovine serum albumin and stored at -80°C. IL-27 was then added to the cells at 100 ng/ml. Type I IFN or IL-27 was added to the cells 24 h postinfection, 24 h before cell surface staining or ADCC assays.

Antibodies and sera. The following antibodies (Abs) were used as the primary Abs for cell surface staining: 5 μ g/ml of human anti-HIV-1 Env monoclonal antibody (MAb) 2G12 (National Institutes of Health [NIH] AIDS and Research and Reference Reagent Program), 2 μ g/ml rabbit anti-BST-2 Ab (sc-99191; Santa Cruz), or sera from HIV-1-infected individuals (1:1,000 dilution), whereas 1 μ g/ml of either Alexa Fluor 647-labeled goat anti-human MABs (Invitrogen, San Diego, CA, USA) or Brilliant Violet 421-labeled donkey anti-rabbit MABs (BioLegend, San Diego, CA, USA) were used as secondary Abs, and AquaVivid (Invitrogen, San Diego, CA, USA) was used as a viability dye. All sera were heat inactivated for 30 min at 56°C and stored at 4°C until ready to use in subsequent experiments. Written informed consent was obtained from all study participants (the Montreal Primary HIV Infection Cohort [53, 54] and the Canadian Cohort of HIV Infected Slow Progressors [55–57]), and research adhered to the ethical guidelines of Centre de Recherche du CHUM (CRCHUM) and was reviewed and approved by the CRCHUM institutional review board (ethics committee). A random-number generator (QuickCalcs; GraphPad) was used to randomly select a number of sera for experiments.

Plasmids. The plasmid encoding the HIV-1 transmitted founder (T/F) CH58 was previously described (5, 17, 58–60).

Flow cytometry analysis of cell surface staining and ADCC responses. Cell surface staining was performed as previously described (1, 5). Binding of HIV-1-infected cells by HIV+ sera, anti-Env MAbs (2G12) or anti-BST-2 MAbs was performed 48 h after infection, 24 h after treatment with type I IFN or IL-27, in the presence or absence of BNM-III-170 (50 μ M) or an equivalent volume of vehicle (DMSO). Detection of p24+ infected cells was performed as described previously (5). The percentage of infected cells (p24+ cells) was determined by gating the living cell population based on the viability dye staining (Aqua Vivid; Invitrogen). Samples were analyzed on a LSRII cytometer (BD Biosciences, Mississauga, ON, Canada), and data analysis was performed using FlowJo vX.0.7 (Tree Star, Ashland, OR, USA).

Measurement of ADCC-mediated killing was performed with a previously described assay (5). Briefly, primary CD4+ T cells infected for 48 h and treated for 24 h with type I IFN or IL-27 or not treated with type I IFN or IL-27 were incubated with autologous PBMCs (effector/target cell ratio of 10:1) in the presence or absence of HIV+ sera (1:1,000), in the presence of CD4mc BNM-III-170 (50 μ M), or with an equivalent volume of vehicle (DMSO). The percentage of cytotoxicity was calculated as described previously (5).

Statistical analyses. Statistics were analyzed using GraphPad Prism version 6.01 (GraphPad, San Diego, CA, USA). Every data set was tested for statistical normality, and this information was used to apply the appropriate (parametric or nonparametric) statistical test. *P* values of <0.05 were considered significant; significance values are indicated as follows: *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001; ****, *P* < 0.0001.

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