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The Envelope Protein of a Human Endogenous Retrovirus-W Family Activates Innate Immunity through CD14/TLR4 and Promotes Th1-Like Responses¹ ✓

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The Envelope Protein of a Human Endogenous Retrovirus-W Family Activates Innate Immunity through CD14/TLR4 and Promotes Th1-Like Responses¹

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Multiple sclerosis-associated retroviral element (MSRV) is a retroviral element, the sequence of which served to define the W family of human endogenous retroviruses. MSRV viral particles display proinflammatory activities both in vitro in human mononuclear cell cultures and in vivo in a humanized SCID mice model. To understand the molecular basis of such properties, we have investigated the inflammatory potential of the surface unit of the MSRV envelope protein (ENV-SU), the fraction that is poised to naturally interact with host cells. We report in this study that MSRV ENV-SU induces, in a specific manner, human monocytes to produce major proinflammatory cytokines through engagement of CD14 and TLR4, which are pattern recognition receptors of primary importance in innate immunity. ENV-SU could also trigger a maturation process in human dendritic cells. Finally, ENV-SU endowed dendritic cells with the capacity to support a Th1-like type of Th cell differentiation. The data are discussed in the context of immune responses and chronic proinflammatory disorders. *The Journal of Immunology*, 2006, 176: 7636–7644.

Human endogenous retroviruses (HERV)⁶ represent ~8% of the human genome and result from integration of exogenous retroviruses that have infected the germline of their host during primate evolution (1). Although most HERV elements are partly or completely deleted following integration, the human genome does contain HERV sequences with open reading frames encoding functional proteins (2). For instance, the so-called W family of HERV possesses a copy on chromosome 7 well known to express an envelope protein called syncytin, which plays an important role in the placenta physiology through syncytiotro-

phoblast formation (3–5). Our knowledge of the possible influence of HERV-encoded components in human disorders remains rather limited. In particular, little is known about the effects of HERV proteins on the innate immune system that represents the first line of defense against viruses and operates largely through detection of invariant microbial molecular patterns by pattern recognition receptors (PRR) expressed on APC, such as monocytes/macrophages and dendritic cells (DC), as well as other cell types. Examples of PRR are the transmembrane TLR that can sense distinct microbial products and are central in innate immune response to various classes of pathogens (6–9).

Multiple sclerosis-associated retroviral element (MSRV) is an enveloped virus with reverse-transcriptase activity (10) that represents the prototype genome that defined the HERV-W family in human DNA (3, 11) and was initially isolated in cell cultures from patients affected by the severe inflammatory demyelinating disorder of the CNS multiple sclerosis (12, 13). The origin of MSRV particles is still unclear. They could originate from a modified endogenous HERV-W provirus or from a transmissible exogenous member of the same family (11, 14, 15). It was observed previously that MSRV virions trigger the secretion of IL-6 and TNF- α proinflammatory cytokines by human PBMC in culture (16). In addition, in SCID mice grafted with human peripheral blood cells, i.p. injected MSRV virion caused an overexpression of TNF- α , leading to death by brain hemorrhages within few days (17). Thus, MSRV particles exert potent proinflammatory effects both in mononuclear cells in culture and in vivo in a humanized SCID mice model.

In the present study, we have investigated the mechanisms of the proinflammatory properties of the surface unit (ENV-SU) of MSRV envelope protein. This fraction contains the binding site to the cellular receptor and allows the virus to naturally interact with host cells. We report that ENV-SU is able to specifically activate cells of the innate immune system, such as monocytes, through PRR CD14 and TLR4. This activation is associated with the production of major proinflammatory cytokines such as IL-1 β , IL-6,

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⁶ Abbreviations used in this paper: HERV, human endogenous retrovirus; DC, dendritic cell; ENV-SU, surface unit of MSRV envelope protein; LAL, *Limulus* amoebocyte lysate; MDDC, monocyte-derived DC; MSRV, multiple sclerosis-associated retroviral element; PB, polymyxin B; PRR, pattern recognition receptor; RSV, respiratory syncytial virus; SEB, staphylococcal enterotoxin B; Treg, regulatory T cell.

or TNF- α . Moreover, we also show that ENV-SU can activate DC and promote the development of Th1-like responses.

Materials and Methods

Proteins and toxins

ENV-SU is a 33-kDa and 293-aa fraction of the full-length MSRV envelope protein (ENV pV14; GenBank accession no. AF331500). ENV-SU and ENV pV14 organizations are presented in Fig. 1A, and amino acid sequence of ENV-SU is presented in Fig. 1B. rENV-SU protein was produced and purified by Protein' eXpert by using the pET-15b expression vector (Novagen) and AD494 (DE3) *Escherichia coli* cells. Briefly, isopropyl- β -D-thiogalactopyranoside-treated cultures grown at 37°C were centrifuged and the pellet was washed. After centrifugation, the pellet was resuspended in wash buffer in the presence of a mixture of protease inhibitors (Roche Diagnostics) and subjected to sonication. The cell lysate was then centrifuged, and the pellet, containing the inclusion bodies, was resuspended in buffer plus protease inhibitors. After centrifugation, the pellet was washed in the presence of 2% Triton X-100, then washed again to remove detergent. Inclusion bodies were solubilized by resuspension in the presence of 8 M urea before centrifugation. The supernatant was diluted in Tris-HCl (pH 8), 1 mM 2-ME, and loaded onto a Chelating Sepharose Fast Flow column (Amersham Biosciences) for purification under denaturing conditions. ENV-SU was refolded by dialysis against 50 mM Tris (pH 7), 300 mM NaCl, 1 mM 2-ME, 2% sucrose, 2% glycerol, and 2 M urea. Under these conditions, the protein remained soluble. Amino acid sequence determination of purified ENV-SU based on Edman degradation was in accordance with the known sequence. Fifty-microgram aliquots/per milliliter were flash frozen into liquid nitrogen and stored at -80°C. SDS-PAGE and mass spectrometry (MALDI-TOF) analysis profiles of rENV-SU (Protein' eXpert) are shown in Fig. 1, C and D. A mock protein, casein kinase, was used as negative control in experiments and was synthesized and purified under the same conditions as ENV-SU. Both proteins were tested for the presence of endotoxins by a *Limulus* amoebocyte lysate (LAL) test performed by CleanCells, and all fractions were below the detection level of 5 UI/ml. Staphylococcal enterotoxin B (SEB) was obtained from Toxin Technology and was 95% pure. LPS from *E. coli* strain 026:B6 was obtained from Sigma-Aldrich.

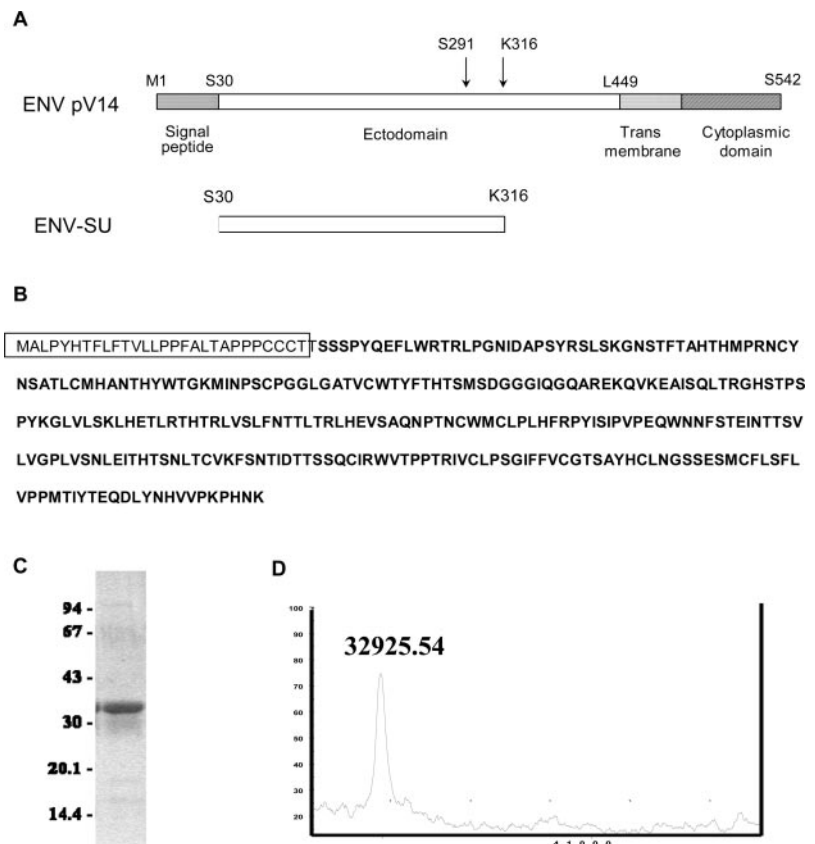
Cell isolation and preparation

Human PBMC were isolated from healthy donor buffy coats by density gradient centrifugation over Ficoll-Paque. Unlabeled monocytes were isolated from PBMCs by depletion of T cells, B cells, DC, basophils, and NK cells by using a Monocyte Isolation Kit II from Miltenyi Biotec. Briefly, cells were incubated with a mixture of biotinylated Abs against CD3, CD7, CD16, CD19, CD56, CD123, and CD235a before addition of anti-biotin MicroBeads. The cell suspension was applied onto a MACS column placed in the magnetic field of a MACS separator, and the effluent was collected along with fractions corresponding to three washes. The purity of monocytes was assessed by immunostaining (anti-CD14 MOP9 mAb) and flow cytometry and ranged from 96 to 98%. For the generation of monocyte-derived DC (MDDC), purified monocytes were cultured for 5 days in the presence of rIL-4 (25 ng/ml) and GM-CSF (100 ng/ml) (both obtained from R&D Systems). Cells were fed at day 3 of culture with full amount of cytokines. As assessed by morphology and flow cytometry analysis, the resulting cell preparation contained >90% of CD1a-positive DC. Similar results were obtained in experiments when MDDC culture was used directly or after depletion of possible residual CD14⁺ cells by immunomagnetic separation. Naive CD4⁺CD45RA⁺ T cells were isolated from total PBMC using a naive T cell enrichment mixture (StemCell Technologies). The recovered CD4⁺ T cell populations were always >90% pure.

Cell stimulation

Cells (PBMC, monocytes, or MDDC) were plated in 24-well plates at a concentration of 1×10^6 /well in 1 ml of medium consisting of RPMI 1640 (Invitrogen Life Technologies) supplemented with 1% L-glutamine, 1% penicillin/streptomycin, 1% sodium-pyruvate, 1% nonessential amino acids (all from Sigma-Aldrich), and 10% heat-inactivated FCS (BioWest). After stimulation with ENV-SU, LPS, SEB, or mock, cells were incubated at 37°C in 5% CO₂ in humidified atmosphere for various periods of time. Where indicated, proteins and toxins were pretreated for 30 min at 37°C with 25 μ g/ml polymyxin B (PB; Sigma-Aldrich) before stimulation. In some cases, cells were preincubated before stimulation with either 20 μ g/ml anti-CD14 (polyclonal sheep IgG; R&D Systems), anti-TLR4 (monoclonal mouse IgG2a; clone HTA125; eBioscience) (18, 19), or anti-TLR2 Abs (monoclonal mouse IgG2a; clone TL2.1; eBioscience). In some

FIGURE 1. ENV-pV14 and ENV-SU organizations and amino acid sequences. *A*, Organizations of ENV-pV14 (full-length MSRV envelope protein) and ENV-SU. ENV-SU is a 293-aa fraction, representing the extracellular surface unit cleaved at K316 of the full-length ENV pV14 protein. *B*, Amino acid sequence of ENV-SU (bold characters). *C*, SDS-12.5% PAGE analysis of rENV-SU after purification by affinity chromatography under denaturing conditions (molecular mass is in kDa). *D*, Mass spectrum of the rENV-SU protein. The profile represents the transformation of the mass to charge ratio on the real mass scale. Vertical axis, percentage of relative intensity; horizontal axis, m.w.



experiments, ENV-SU, mock, LPS, and SEB were boiled for 30 min before cell stimulation. To determine the specificity of our results, before cell stimulation, 1 μg of ENV-SU, mock, and LPS was preincubated for 45 min at 4°C with 30 $\mu\text{g}/\text{ml}$ mAbs directed against either ENV-SU (monoclonal mouse IgG1; clone 13H5A5 and 3B2H4; bioMérieux) or GAG (monoclonal mouse IgG1; clone 3H1H6; bioMérieux) recombinant proteins. The 13H5A5, 3B2H4, and 3H1H6 mAbs were obtained by immunization of mice with, respectively, ENV-SU and GAG recombinant proteins. The specificity of the Abs was controlled by ELISA, and only 13H5A5 and 3B2H4 mAbs specifically bound ENV-SU recombinant protein.

Immunofluorescence staining and flow cytometry

Cells were harvested, washed in PBS, and immunostained for surface expression of distinct markers. The following mAbs were obtained from BD Biosciences: anti-CD1a allophycocyanin (HI149-allophycocyanin), anti-CD14 FITC (MOP9-FITC), CD40 PE (5C3-PE), CD80 PE (L307.4-PE), CD86 PE (IT2.2-PE), and HLA-DR PerCP (L243-PerCP). Briefly, direct immunofluorescence staining of cells was performed in ice-cold PBS supplemented with 2% of FCS in the presence of mAbs at concentrations recommended by the manufacturers. After 30 min at 4°C, cells were washed and analyzed using a FACSCalibur and the software CellQuest (BD Biosciences).

Cytokine production and T cell polarization assays

Culture supernatants were harvested and preserved at -20°C before evaluation of cytokine production by ELISA. OptEIA ELISA kits from BD Pharmingen for detection of human IL-1 β , IL-6, IL-12p40, IL-12p70, and TNF- α were used, according to the manufacturer's instructions.

For T cell polarization assay, stimulated MDDC were used as stimulators. Responder cells were purified allogeneic CD4⁺CD45RA⁺ T cells used at $1 \times 10^5/\text{well}$ (96-well round-bottom microtiter plates). Stimulatory cells were added to T cells in graded doses, and cultures were set up in triplicates in a final volume of 200 μl of medium supplemented with 10% of human AB serum (Sigma-Aldrich). After 5 days of incubation at 37°C, cell supernatants were collected and tested for the production of T cell cytokines by ELISA.

Results

ENV-SU stimulates the production of proinflammatory cytokines in human PBMC cultures

We first studied ENV-SU abilities to stimulate cytokine production in PBMC cultures from healthy controls. Human PBMC were incubated with graded doses of ENV-SU for 24 h, and secretions of TNF- α , IL-1 β , and IL-6 were analyzed by ELISA. We observed

that ENV-SU induced the production of all three cytokines in a dose-dependent manner even at doses as low as 10 ng/ml (Fig. 2A). In parallel, a mock control protein (mock), produced and purified under the same conditions as ENV-SU, was also tested and no cytokine production was observed (data not shown). We then studied the kinetics of cytokine secretion induced by ENV-SU in PBMC cultures and compared it with mock, SEB (a well-characterized bacterial superantigen), and LPS. All proteins and toxins were used at a concentration of 1 $\mu\text{g}/\text{ml}$, found to be the optimal concentration for proinflammatory cytokine production. As shown in Fig. 2B, ENV-SU-mediated kinetics of cytokine secretion was more similar to that of LPS rather than SEB. Both ENV-SU and LPS induced the secretion of high amounts of TNF- α , IL-6, and IL-1 β already after 24 h. TNF- α and IL-1 β reached their peak of production at this time point and then decreased, while IL-6 levels constantly increased following stimulation. SEB did not induce any IL-1 β or IL-6, but a constant TNF- α secretion was observed. Mock-induced cytokines remained below the detection threshold. Finally, separate experiments have revealed that secretion of IFN- γ and IL-10 is marginal in PBMC cultures treated for 24 h with soluble ENV-SU (20). Thus, ENV-SU induces the secretion of major proinflammatory cytokines in human PBMC cultures from healthy donors.

ENV-SU-mediated proinflammatory properties are specifically inhibited by mAbs and are not due to endotoxin contaminations

The possibility that the proinflammatory effect of ENV-SU essentially reflected endotoxin contamination was ruled out by successive analysis. As a first control, the protein preparations were tested in a Food and Drug Administration-approved LAL assay (see *Materials and Methods*) and were negative. Indeed, it was unlikely that trace amounts (<5 UI/ml) of endotoxin could cause a higher release of IL-6 by PBMC than the secretion observed in the presence of 1 $\mu\text{g}/\text{ml}$ pure LPS (Fig. 2B), and doses of LPS corresponding to the detection threshold of the LAL assay effectively did not cause such effects (data not shown). To establish the specificity of ENV-SU proinflammatory properties, we studied the

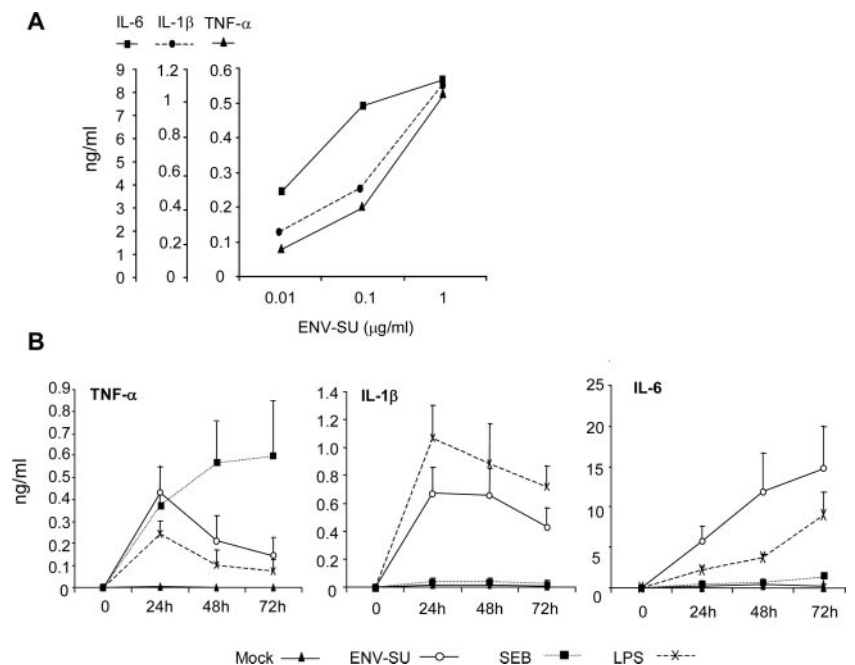
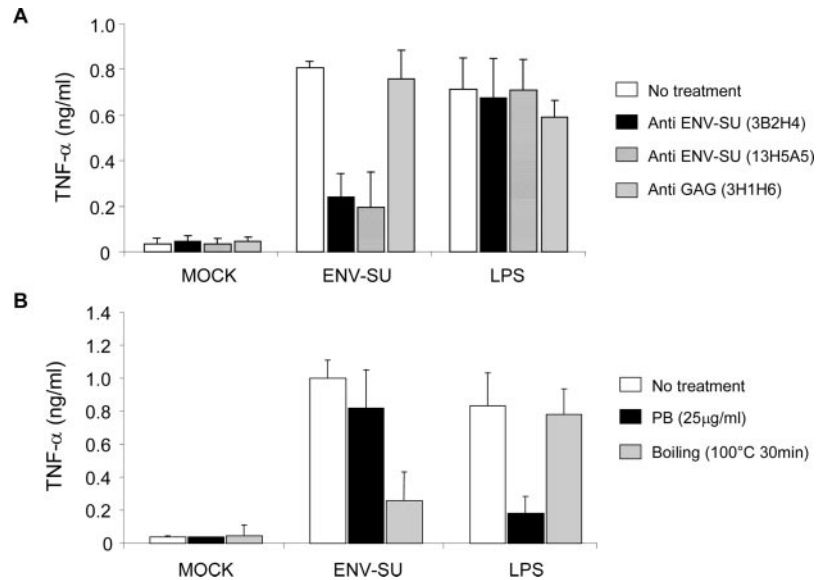


FIGURE 2. ENV-SU induces the production of proinflammatory cytokines in PBMC cultures. *A*, PBMC from healthy donors were stimulated for 24 h with graded doses of ENV-SU, and culture supernatants were then analyzed by ELISA for TNF- α , IL-1 β , and IL-6 production. *B*, PBMC were stimulated with 1 $\mu\text{g}/\text{ml}$ mock, ENV-SU, LPS, or SEB and incubated for 24, 48, and 72 h before analysis of cytokine secretion by ELISA. Results are presented as the mean \pm SE of three independent experiments.

FIGURE 3. ENV-SU-mediated TNF- α production is inhibited by specific mAbs and is not due to endotoxin contamination. *A*, PBMC were stimulated for 24 h with 1 μ g/ml mock control, ENV-SU, and LPS preincubated or not for 45 min at 4°C with mAbs (30 μ g/ml) specific for ENV-SU (clones 13H5H5 and 3B2H4) or GAG (clone 3H1H6) proteins. Culture supernatants were harvested and analyzed for TNF- α secretion. *B*, Where indicated, the proteins and toxins were pretreated for 30 min at 37°C with 25 μ g/ml PB before PBMC stimulation. In parallel, cells were also incubated with proteins and toxins boiled for 30 min (100°C). Culture supernatants were then harvested and tested by ELISA for TNF- α release. Results represent the mean \pm SE of three independent experiments.



effects of anti-ENV-SU mAbs on the cytokine production previously described. PBMC were incubated for 24 h with mock, ENV-SU, or LPS preincubated with Abs directed against either MSR V ENV-SU or MSR V GAG. As shown in Fig. 3A, two of the anti-ENV-SU mAbs (13H5A5 and 3B2H4) tested specifically blocked ENV-SU-mediated TNF- α secretion, but not that induced by LPS. In contrast, cytokine secretion was not affected in either case by treatment with anti-GAG mAb. Because the 13H5A5 and 3B2H4 mAbs had no effect on LPS-induced TNF- α secretion, the data make the additional point that the inhibition of ENV-SU proinflammatory activity cannot be explained by a hypothetical toxic effect of these Abs on PBMC. These results definitely demonstrate that ENV-SU can specifically cause PBMC activation and cytokine release.

Nevertheless, we performed complementary control experiments either by boiling or treating the proteins and toxins with the cationic antibiotic PB that neutralizes LPS activity, before their addition to the culture. Fig. 3B shows that while PB treatment completely abolished LPS-induced TNF- α secretion, it only marginally affected ENV-SU-induced TNF- α production. Conversely,

boiled ENV-SU lost most of its stimulatory capacity, whereas LPS stimulatory potential remained unaffected by boiling. Taken together, the results indicate that residual endotoxin contamination cannot account for the proinflammatory activity of ENV-SU and that the component responsible for this activity is a protein.

ENV-SU directly activates purified human monocytes

While analyzing total PBMC cultures exposed to ENV-SU for 24 and 48 h, we observed that the frequency of both CD25⁺ and CD69⁺ T lymphocytes remained at the background level. By intracellular staining, CD3⁺ mononuclear cells were also devoid of TNF- α , IFN- γ , and IL-4 production (data not shown). Thus, the data failed to reveal any signs of T cell activation in response to soluble ENV-SU in short-term cultures. In addition, the profile of cytokine production induced by ENV-SU was comparable to that induced by LPS and is reminiscent of the pattern observed upon monocyte activation. We therefore examined whether ENV-SU was able to directly activate monocytes. Unmanipulated CD14⁺ mononuclear cells isolated by negative immunomagnetic separation (>96% purity) were stimulated with mock, ENV-SU, or LPS

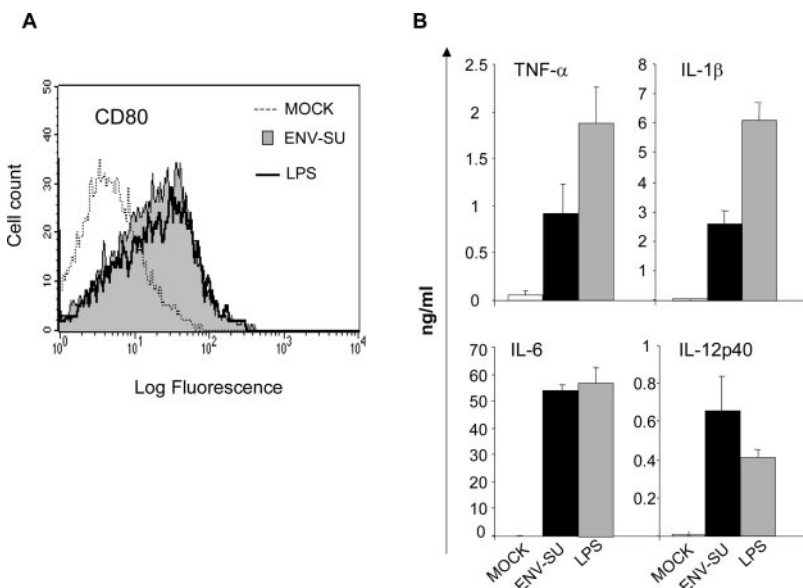


FIGURE 4. ENV-SU directly activates purified human monocytes. *A*, Human monocytes were purified from human PBMC (>95% purity) and then stimulated with mock, ENV-SU, or LPS at 1 μ g/ml for 24 h. Cells were harvested, and surface expression of the activation marker CD80 was analyzed by flow cytometry. One representative experiment of mock, ENV-SU, and LPS stimulation is presented. *B*, Cytokine production (TNF- α , IL-1 β , IL-6, and IL-12p40) was analyzed by ELISA. Results represent the mean \pm SE of three independent experiments.

for 24 h, and the expression of the activation marker CD80 was evaluated by flow cytometry. When compared with mock, ENV-SU induced the up-regulation of CD80 expression at a level similar to that induced by LPS (Fig. 4A). Several other markers such as CD40, CD86, and HLA-DR were also tested, but no differences were observed at this time point (data not shown). We then studied the profile of cytokine secretion induced by ENV-SU in purified monocytes and observed that high amounts of TNF- α , IL-1 β , IL-6, and IL-12p40 were produced in response to ENV-SU (Fig. 4B), while only marginal levels of IL-12p70 were obtained (data not shown). Similar observations were made when using heat-inactivated MSRV particles, indicating that the proinflammatory effects of rENV-SU on purified monocytes are recapitulated by viral particles (Fig. 5). Taken together, these observations indicate that ENV-SU induces a rapid and direct monocyte activation associated with proinflammatory cytokine secretion.

CD14 and TLR4 PRR are involved in the proinflammatory response to ENV-SU

The detection of pathogen-associated molecular patterns by APCs relies largely on a group of transmembrane PRR named TLR (6, 7, 9). For instance, TLR4 engagement mediates monocyte/macrophage activation in response to Gram-negative bacteria through LPS detection (21). Findings supporting a role for TLR in virus detection include the sensing of viral dsRNA by TLR3 (22), a role for TLR2 in IL-6 secretion by macrophages in response to the measles virus (23), and the identification of TLR4 as an essential component of the response to respiratory syncytial virus (RSV) fusion protein (24) and possibly mouse mammary tumor virus (25). To determine whether ENV-SU is susceptible to engage activation pathways similar to those triggered by LPS or RSV fusion protein (namely TLR4 and the accessory glycosyl phosphatidylinositol-anchored protein CD14), we pretreated purified monocytes

with anti-CD14, anti-TLR4, and TLR2 neutralizing Abs before activation with ENV-SU, LPS, or PMA, which activates monocytes in a TLR4/CD14-independent manner. The levels of TNF- α secreted were then measured (Fig. 6). No significant inhibition of TNF- α production was observed with any blocking Ab tested when monocytes were stimulated with PMA. In contrast, when tested on ENV-SU- or LPS-stimulated monocytes, both CD14 and TLR4 blocking Abs caused substantial inhibition of TNF- α secretion. The inhibition of ENV-SU-induced cytokine production by anti-CD14 Abs was consistently stronger than that observed in the presence of the weak affinity anti-TLR4 HTA125 Ab. Such inhibitory effects were found to be dose sensitive (data not shown). TLR2 blocking Ab, which was used as an isotype control, did not induce any inhibition under any conditions tested. In addition, TLR4, but not TLR2, blocking Ab was also shown to interfere with cytokine production induced by heat-inactivated MSRV particles (Fig. 7). These results indicate that both TLR4 and CD14 PRR are involved in the proinflammatory effects of ENV-SU on human monocytes.

ENV-SU directly activates MDDC and confers them the potential to support the development of Th1-like responses

DC are professional APCs that express a variety of TLRs. TLR engagement induces DC to up-regulate Ag-presenting and costimulatory molecules expression as well as to secrete proinflammatory cytokines. These maturation events render them

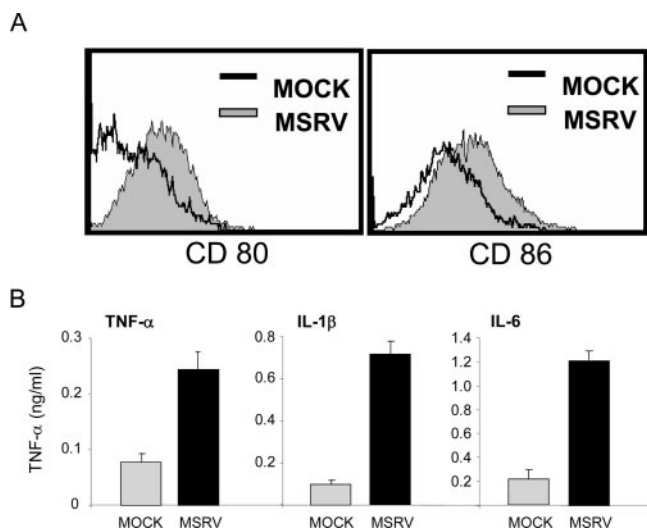


FIGURE 5. MSRV particles directly activate purified monocytes. *A*, Purified monocytes were stimulated for 24 h with 2×10^3 MSRV particles or mock control, and the expression of several activation markers such as CD80 and CD86 was assessed by flow cytometry. The surface expression of both CD80 and CD86 was up-regulated following MSRV treatment. MSRV particles were obtained from MSRV-infected immortalized B cell cultures after ultracentrifugation. MSRV particles were quantified by reverse-transcriptase activity measurement. All MSRV particle preparations were mycoplasma free. The mock control was prepared from MSRV-negative B cell cultures. *B*, The supernatants were then tested for proinflammatory cytokine secretion by ELISA. The values are the mean \pm SE of replicate from one experiment representative of three.

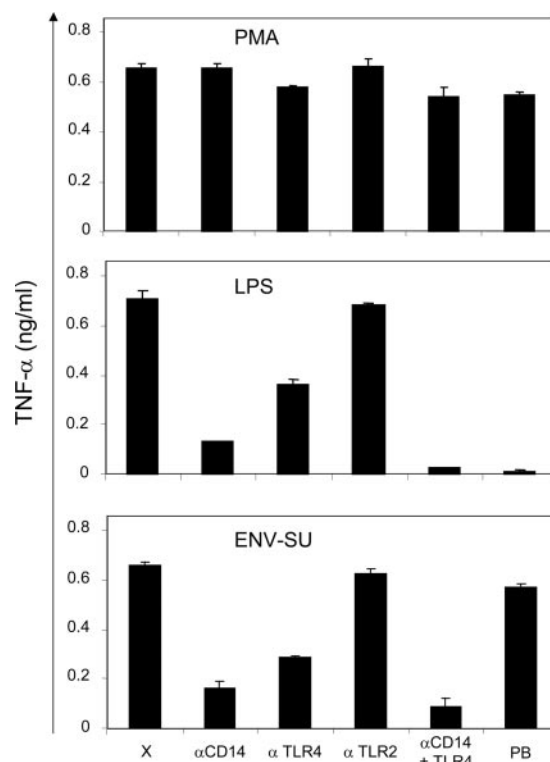


FIGURE 6. CD14 and TLR4 are involved in ENV-SU-mediated proinflammatory properties. Purified monocytes were preincubated at 37°C for 1 h with or without neutralizing Abs to CD14 (polyclonal IgG), TLR4 (HRTA125 mAb, functional grade), and TLR2 (TL2.1 mAb) (all at 20 μ g/ml), and then further stimulated for 24 h with PMA at 100 ng/ml, ENV-SU at 200 ng/ml, or LPS at 50 ng/ml. The effects of PB were also tested in parallel as a control. Culture supernatants were harvested and TNF- α release was evaluated by ELISA. Isotype controls (functional grade mouse IgG2a and polyclonal sheep IgG) were also tested and no inhibitory effects were observed (data not shown). The values represent the mean \pm SE of replicate from one experiment representative of three.

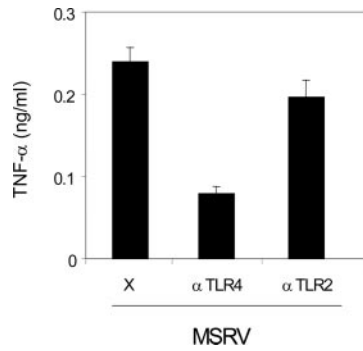


FIGURE 7. MSRV particles activate purified monocytes through TLR4. Purified monocytes were preincubated at 37°C for 1 h with or without (X) neutralizing Abs to TLR4 (α TLR4) or TLR2 (α TLR2) (both at 20 μ g/ml) and then further stimulated for 24 h with 2×10^3 MSRV particles. Culture supernatants were harvested, and TNF- α release was evaluated by ELISA. The values correspond to the mean \pm SE of three independent experiments.

highly potent at activating naive T cells and promoting their differentiation in effector cells (26, 27). We therefore examined whether ENV-SU was able to directly activate MDDC and whether these MDDC would show any capacity to polarize naive T cell responses. MDDC were generated in vitro from highly purified human monocytes by using IL-4 and GM-CSF and stimulated during 24 h with mock, ENV-SU, or LPS. In

contrast to the differentiation marker CD1a, whose expression level remained unaltered, we observed that ENV-SU induced a substantial increase in the surface expression level of CD80, CD86, CD40, and HLA-DR molecules, indicating that ENV-SU triggers a phenotypic maturation process in MDDC (Fig. 8A). Regarding the secretion of proinflammatory cytokines, IL-6, TNF- α , IL-12p40, and IL-12p70 were produced at rather high levels only in the presence of ENV-SU or LPS (Fig. 8B).

IL-12 is a major cytokine for committing T cells to Th1 lineage differentiation (28). Th1 and Th2 subsets can develop upon interaction of mature DC with the same T cell precursor, which is a naive CD4 T cell. Thus, to evaluate the ability of stimulated MDDC to polarize naive T lymphocytes, MDDC were cocultured with purified allogeneic CD4⁺CD45RA⁺ T cells, and cytokine production was measured. MDDC pulsed with ENV-SU were much more efficient at inducing naive T cells to secrete IFN- γ than IL-4 (Th1- and Th2-associated cytokines, respectively). High amounts of IFN- γ (Fig. 8C, left panel) were indeed produced even at responder/stimulator ratios as low as 1/100, while the amount of IL-4 (right panel) obtained when MDDC were stimulated with ENV-SU was consistently below the background level of the mock control. By performing inhibition experiments, we found that anti-CD14 Abs could detectably interfere with the phenotypic activation of immature MDDC induced by ENV-SU (Fig. 9A). Inhibition by the anti-TLR4 HTA125 mAb was marginal (data not shown). Nevertheless, we consistently noticed that HTA125 was able to

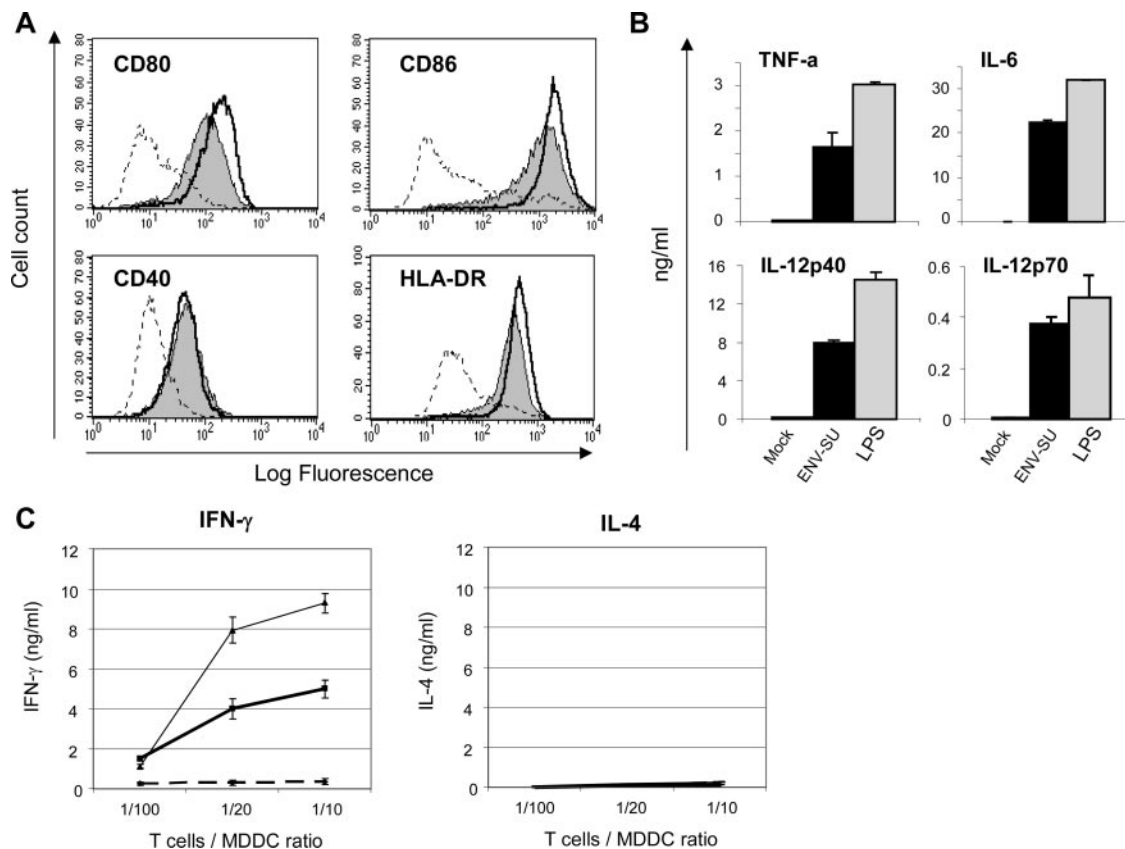


FIGURE 8. MDDC activated with ENV-SU secrete IL-12 and promote the development of Th1-like responses. A, MDDC were generated from purified monocytes and then stimulated with mock, ENV-SU (1 μ g/ml), or LPS (100 ng/ml) for 24 h. Surface staining of activation markers HLA-DR, CD80, CD86, and CD40 was made after mock (dotted line), ENV-SU (shaded histogram), and LPS (bold line) treatments, and then analyzed by flow cytometry. One representative experiment is presented. B, Cytokine productions (TNF- α , IL-6, IL-12p40, and IL-12p70) were analyzed by ELISA. C, Various concentrations of MDDC stimulated either with mock (dotted line), ENV-SU (bold line), or LPS were cocultured for 5 days with purified allogeneic CD4⁺CD45RA⁺ T cells. Supernatants were then tested for IFN- γ (left panel) and IL-4 release (right panel). The values represent the mean \pm SE of three (B) and two (C) independent experiments.

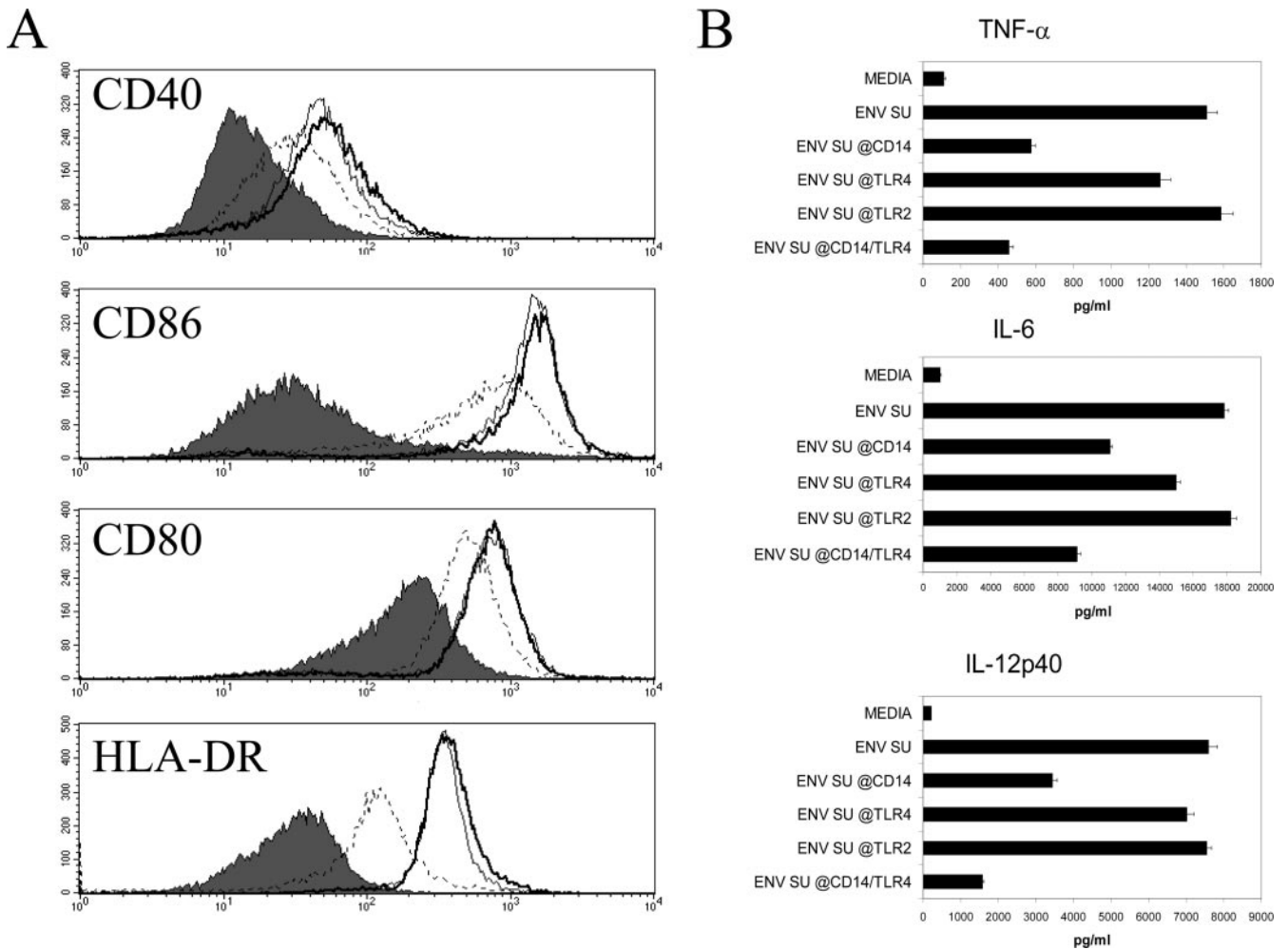


FIGURE 9. CD14 and TLR4 receptors are involved in ENV-SU-mediated activation of immature MDDC. MDDC were stimulated with ENV-SU (5 ng/ml) and analyzed for phenotypic maturation and cytokine secretion. *A*, Inhibition of ENV-SU-induced MDDC phenotypic maturation by anti-CD14 mAbs. Surface expression of CD40, CD86, CD80, and HLA-DR was analyzed by immunostaining and flow cytometry (vertical axis, cell count; horizontal axis, log fluorescence intensity). Vehicle (filled histograms), ENV-SU (open histograms, bold line), ENV-SU + 20 μ g/ml anti-CD14 (open histograms, dotted line), and ENV-SU + 20 μ g/ml control IgG (open histograms, regular line). The histograms shown are representative of three independent experiments. *B*, Additive inhibitory effect of anti-TLR4 and anti-CD14 Abs on cytokine secretion by ENV-SU-treated immature MDDC. Cytokine productions (TNF- α , IL-6, and IL-12p40) were analyzed by ELISA after stimulation in the presence of anti-CD14, anti-TLR4, anti-TLR2, or anti-CD14 + anti-TLR4. The data are representative of two independent experiments.

reinforce the inhibitory effect of the anti-CD14 Abs on ENV-SU-induced cytokine secretion by MDDCs. This effect was indeed reminiscent of that observed in experiments performed with purified monocytes (see Fig. 6). Altogether, the data indicate that ENV-SU is able to induce phenotypic and functional maturation of DC and confers them the potential to support the development of Th1-like effector T lymphocytes.

Discussion

The present study provides evidence that ENV-SU, a retroviral element of the HERV-W family, is capable of activating innate immunity through PRR TLR4/CD14. This conclusion arises from the observation that ENV-SU induced human monocytes to produce major proinflammatory cytokines in a CD14- and TLR4-dependent fashion. The specificity of such phenomenon was unequivocally shown by the inhibitory effect of two anti-MSRV-ENV mAbs. ENV-SU also induced DC maturation and conferred them the capacity to support a Th1 type of T cell differentiation.

CD14 and TLR4 are evolutionary conserved receptors with critical roles for the activation of APCs and proinflammatory cytokine

production in response to LPS (21, 29, 30). In addition, RSV induces cytokine secretion by human monocytes via CD14 and TLR4 engagement, and TLR4-deficient mice infected with RSV showed deficient IL-12 production and NK cell response as well as an increased titer of viral particles in the lung relative to control mice (31). Thus, CD14 and TLR4 are PRR that cooperate for the activation of innate immunity in response to both bacteria and viruses. Our observations that CD14 and TLR4 are involved in mediating the proinflammatory effect of the MSRV envelope protein identify proteins of HERV-W family as a putative new class of viral ligand for these PRR. As DC do not express CD14 at their surface, their capacity to react to MSRV ENV-SU most certainly relies on the recruitment of soluble CD14 that is present in the serum, as it is known to be the case for the response of DC to LPS stimulation (32, 33).

The activation of the innate immune system might contribute to the development of neurodegenerative diseases, and a possible role for CD14 and TLR4 in autoimmune/proinflammatory disorders has been evoked. It was thus shown that the level of soluble CD14

naturally present in plasma was elevated in diseases such as multiple sclerosis (34), rheumatoid arthritis (35), or systemic lupus erythematosus (36). Moreover, specific activation of CNS innate immunity through TLR4 can lead to neurodegenerative phenomena (37) via activation of brain-resident macrophages (microglia), which are the only glial cells with TLR4 expression. Finally, TLR4 is necessary for LPS-induced oligodendrocyte injury in the CNS (38). These results indicate that the engagement of TLR4 expressed on CNS resident, and/or perivascular, macrophages by its ligands might contribute to oligodendrocyte damage and neurodegeneration.

Because MSRV was first isolated in choroid plexus/leptomeningeal cell cultures from multiple sclerosis patients (12, 13), and virion-associated MSRV RNA can be detected in sera and/or cerebrospinal fluids of such patients (39, 40), it is plausible that its envelope protein can exert its CD14/TLR4-dependent proinflammatory effect within the CNS, and therefore initiates and/or substantially exacerbates the disorder. This idea is in line with the findings that: 1) HERV-W ENV and GAG glycoproteins are expressed in the white matter of patients with multiple sclerosis (41–43), and that 2) the virus load detected in the CSF increases with MS progression and thus may have prognosis value (38). HERV-W 7q ENV expression is enhanced relative to tissues from healthy controls or patients with other neurological disorders and has the potential to cause inflammation and oligodendrocyte death through the induction of redox reactants in astrocytes (41).

A role for TLR has also been argued in initiation of autoimmunity. One possible way by which MSRV-mediated activation of the TLR4 signaling pathway could contribute to the development of autoimmunity is by interference with the immune suppression naturally mediated by a subclass of CD4⁺ T lymphocytes named regulatory T cells (Treg). For instance, Treg can readily suppress the development of autoimmune responses in experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis (44). A recent report documented that IL-6 produced following TLR-mediated recognition of microbial products renders naive CD4⁺ T cells insensitive to the suppressive activity of CD4⁺CD25⁺ Treg (45). We have demonstrated in this study that via ENV-SU, MSRV could induce the production of massive amounts of IL-6 in human monocyte and MDDC cultures. Thus, through the TLR4-dependent secretion of IL-6, ENV-SU could interfere with the suppressive activity of Treg cells and, therefore, facilitate the priming of autoreactive T cells.

DC are APCs, which, upon activation, have the unique ability to induce primary specific immune responses. Various pathogen products can induce the maturation of DC through TLR signaling. In the present study, MDDC pulsed with ENV-SU displayed all features of a mature phenotype. Interestingly, in the context of multiple sclerosis, some DC were shown to express the maturation marker CD83 in active lesions (46) and to secrete proinflammatory cytokines such as IL-6 and TNF- α in peripheral blood (47). Depending on the maturation stimuli, MDDC can induce the activation/polarization of naive T cells toward either Th1 or Th2 lineage. MDDC stimulated with ENV-SU produced large amounts of proinflammatory cytokines, including IL-12p70, and were able to promote the development of naive CD4⁺CD45RA⁺ T cells into IFN- γ -secreting Th1-like cells. Induction of Th1 cells insufficiently counterbalanced by Th2 cells has indeed been proposed in the pathogenesis of demyelinating disorders (48).

Altogether, our observations support the notion that ENV-SU of the HERV-W family MSRV element can activate the innate immune system through a TLR4/CD14-dependent pathway and is susceptible to promote the development of a Th1 type of immune

response upon DC activation. The data are compatible with the idea that, through the proinflammatory properties of its surface envelope protein, MSRV could be involved in the immunopathological cascades associated with chronic inflammatory and/or neurodegenerative diseases.

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Disclosures

A. Rolland, E. Jouvin-Marche, H. Perron, and P. N. Marche are inventors on a patent for the anti-Envelope mAbs as inhibitors of the Envelope–TLR4 interaction. Also involved with this patent are bioMérieux SA, and Institut National de la Santé et de la Recherche Médicale.

References

1. Belshaw, R., V. Pereira, A. Katzourakis, G. Talbot, J. Paces, A. Burt, and M. Tristem. 2004. Long-term reinfection of the human genome by endogenous retroviruses. *Proc. Natl. Acad. Sci. USA* 101: 4894–4899.
2. Lower, R., J. Lower, and R. Kurth. 1996. The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. *Proc. Natl. Acad. Sci. USA* 93: 5177–5184.
3. Blond, J. L., F. Beseme, L. Duret, O. Bouton, F. Bedin, H. Perron, B. Mandrand, and F. Mallet. 1999. Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family. *J. Virol.* 73: 1175–1185.
4. Blond, J. L., D. Lavillette, V. Cheynet, O. Bouton, G. Oriol, S. Chapel-Fernandes, B. Mandrand, F. Mallet, and F. L. Cosset. 2000. An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. *J. Virol.* 74: 3321–3329.
5. Mi, S., X. Lee, X. Li, G. M. Veldman, H. Finnerty, L. Racie, E. LaVallie, X. Y. Tang, P. Edouard, S. Howes, et al. 2000. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403: 785–789.
6. Janeway, C. A., Jr., and R. Medzhitov. 2002. Innate immune recognition. *Annu. Rev. Immunol.* 20: 197–216.
7. Medzhitov, R. 2001. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* 1: 135–145.
8. Janeway, C. A., Jr. 1989. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harbor Symp. Quant. Biol.* 1: 1–13.
9. Akira, S. 2003. Mammalian Toll-like receptors. *Curr. Opin. Immunol.* 15: 238.
10. Perron, H., J. A. Garson, F. Bedin, F. Beseme, G. Paranhos-Baccala, F. Komurian-Pradel, F. Mallet, P. W. Tuke, C. Voisset, J. L. Blond, et al. 1997. Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis: the Collaborative Research Group on Multiple Sclerosis. *Proc. Natl. Acad. Sci. USA* 94: 7583–7588.
11. Komurian-Pradel, F., G. Paranhos-Baccala, F. Bedin, A. Ounanian-Paraz, M. Sodoyer, C. Ott, A. Rajoharison, E. Garcia, F. Mallet, B. Mandrand, and H. Perron. 1999. Molecular cloning and characterization of MSRV-related sequences associated with retrovirus-like particles. *Virology* 260: 1–9.
12. Perron, H., C. Geny, A. Laurent, C. Mouriquand, J. Pellat, J. Perret, and J. M. Seigneurin. 1989. Leptomeningeal cell line from multiple sclerosis with reverse transcriptase activity and viral particles. *Res. Virol.* 140: 551–561.
13. Perron, H., B. Lalande, B. Gratacap, A. Laurent, O. Genoulaz, C. Geny, M. Mallaret, E. Schuller, P. Stoebner, and J. M. Seigneurin. 1991. Isolation of retrovirus from patients with multiple sclerosis. *Lancet* 337: 862–863.
14. Perron, H., J. P. Perin, F. Rieger, and P. M. Alliel. 2000. Particle-associated retroviral RNA and tandem *RGH/HERV-W* copies on human chromosome 7q: possible components of a “chain-reaction” triggered by infectious agents in multiple sclerosis? *J. Neurovirol.* 2(Suppl. 6): S67–S75.
15. Perron, H., B. Gratacap, B. Lalande, O. Genoulaz, A. Laurent, C. Geny, M. Mallaret, P. Innocenti, E. Schuller, P. Stoebner, et al. 1992. In vitro transmission and antigenicity of a retrovirus isolated from a multiple sclerosis patient. *Res. Virol.* 143: 337–350.
16. Perron, H., E. Jouvin-Marche, M. Michel, A. Ounanian-Paraz, S. Camelo, A. Dumon, C. Jolivet-Reynaud, F. Marcel, Y. Souillet, E. Borel, et al. 2001. Multiple sclerosis retrovirus particles and recombinant envelope trigger an abnormal immune response in vitro, by inducing polyclonal V β 16 T-lymphocyte activation. *Virology* 287: 321–332.
17. Firouzi, R., A. Rolland, M. Michel, E. Jouvin-Marche, J. J. Hauw, C. Malcus-Vocanson, F. Lazarini, L. Gebuhrer, J. M. Seigneurin, J. L. Touraine, et al. 2003. Multiple sclerosis-associated retrovirus particles cause T lymphocyte-dependent death with brain hemorrhage in humanized SCID mice model. *J. Neurovirol.* 9: 79–93.
18. Shimazu, R., S. Akashi, H. Ogata, Y. Nagai, K. Fukudome, K. Miyake, and M. Kimoto. 1999. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J. Exp. Med.* 189: 1777–1782.
19. Wang, R., J. Stephens, and M. J. Lacy. 2003. Characterization of monoclonal antibody HTA125 with specificity for human TLR4. *Hybrid Hybridomics* 22: 357–365.

20. Rolland, A., E. Jouvin-Marche, M. Saresella, P. Ferrante, R. Cavaretta, A. Creange, P. Marche, and H. Perron. 2005. Correlation between disease severity and in vitro cytokine production mediated by MSRV (multiple sclerosis associated retroviral element) envelope protein in patients with multiple sclerosis. *J. Neuroimmunol.* 160: 195–203.
21. Poltorak, A., X. He, I. Smirnova, M. Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, et al. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282: 2085–2088.
22. Alexopoulou, L., A. C. Holt, R. Medzhitov, and R. A. Flavell. 2001. Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* 413: 732–738.
23. Bieback, K., E. Lien, I. M. Klagge, E. Avota, J. Schneider-Schaulies, W. P. Duprex, H. Wagner, C. J. Kirschning, V. Ter Meulen, and S. Schneider-Schaulies. 2002. Hemagglutinin protein of wild-type measles virus activates Toll-like receptor 2 signaling. *J. Virol.* 76: 8729–8736.
24. Kurt-Jones, E. A., L. Popova, L. Kwinn, L. M. Haynes, L. P. Jones, R. A. Tripp, E. E. Walsh, M. W. Freeman, D. T. Golenbock, L. J. Anderson, and R. W. Finberg. 2000. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol.* 1: 398–401.
25. Rassa, J. C., J. L. Meyers, Y. Zhang, R. Kudaravalli, and S. R. Ross. 2002. Murine retroviruses activate B cells via interaction with Toll-like receptor 4. *Proc. Natl. Acad. Sci. USA* 99: 2281–2286.
26. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nat. Med.* 5: 868–870.
27. Palucka, K., and J. Banchereau. 1999. Linking innate and adaptive immunity. *Nat. Med.* 5: 868–870.
28. Trinchieri, G., S. Pflanz, and R. A. Kastelein. 2003. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity* 19: 641–644.
29. Hoshino, K., O. Takeuchi, T. Kawai, H. Sanjo, T. Ogawa, Y. Takeda, K. Takeda, and S. Akira. 1999. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J. Immunol.* 162: 3749–3752.
30. Qureshi, S. T., L. Lariviere, G. Leveque, S. Clermont, K. J. Moore, P. Gros, and D. Malo. 1999. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J. Exp. Med.* 189: 615–625.
31. Haynes, L. M., D. D. Moore, E. A. Kurt-Jones, R. W. Finberg, L. J. Anderson, and R. A. Tripp. 2001. Involvement of Toll-like receptor 4 in innate immunity to respiratory syncytial virus. *J. Virol.* 75: 10730–10737.
32. Frey, E. A., D. S. Miller, T. G. Jahr, A. Sundan, V. Bazil, T. Espevik, B. B. Finlay, and S. D. Wright. 1992. Soluble CD14 participates in the response of cells to lipopolysaccharide. *J. Exp. Med.* 176: 1665–1671.
33. Verhasselt, V., C. Buelens, F. Willems, D. De Groote, N. Haeflner-Cavaillon, and M. Goldman. 1997. Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells: evidence for a soluble CD14-dependent pathway. *J. Immunol.* 158: 2919–2925.
34. Bretschneider, J., D. Ecker, A. Bitsch, D. Bahner, T. Bogumil, A. Dressel, E. Elitok, B. Kitze, S. Poser, F. Weber, and H. Tumani. 2002. The macrophage activity marker sCD14 is increased in patients with multiple sclerosis and up-regulated by interferon β -1b. *J. Neuroimmunol.* 133: 193–197.
35. Yu, S., N. Nakashima, B. H. Xu, T. Matsuda, A. Izumihara, N. Sunahara, T. Nakamura, M. Tsukano, and T. Matsuyama. 1998. Pathological significance of elevated soluble CD14 production in rheumatoid arthritis: in the presence of soluble CD14, lipopolysaccharides at low concentrations activate RA synovial fibroblasts. *Rheumatol. Int.* 17: 237–243.
36. Nockher, W. A., R. Wigand, W. Schoeppe, and J. E. Scherberich. 1994. Elevated levels of soluble CD14 in serum of patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* 96: 15–19.
37. Lehnardt, S., L. Massillon, P. Follett, F. E. Jensen, R. Ratan, P. A. Rosenberg, J. J. Volpe, and T. Vartanian. 2003. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proc. Natl. Acad. Sci. USA* 100: 8514–8519.
38. Lehnardt, S., C. Lachance, S. Patrizi, S. Lefebvre, P. L. Follett, F. E. Jensen, P. A. Rosenberg, J. J. Volpe, and T. Vartanian. 2002. The Toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. *J. Neurosci.* 22: 2478–2486.
39. Dolei, A., C. Serra, G. Mamei, M. Pugliatti, G. Sechi, M. C. Ciroto, G. Rosati, and S. Sotgiu. 2002. Multiple sclerosis-associated retrovirus (MSRV) in Sardinian MS patients. *Neurology* 58: 471–473.
40. Sotgiu, S., C. Serra, G. Mamei, M. Pugliatti, G. Rosati, G. Arru, and A. Dolei. 2002. Multiple sclerosis-associated retrovirus and MS prognosis: an observational study. *Neurology* 59: 1071–1073.
41. Antony, J. M., G. van Marle, W. Opri, D. A. Butterfield, F. Mallet, V. W. Yong, J. L. Wallace, R. M. Deacon, K. Warren, and C. Power. 2004. Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. *Nat. Neurosci.* 7: 1088–1095.
42. Perron, H., F. Lazarini, K. Ruprecht, C. Pechoux-Longin, D. Seilhean, V. Sazdovitch, A. Creange, N. Battail-Poirot, G. Sibai, L. Santoro, et al. 2005. Human endogenous retrovirus (HERV)-W ENV and GAG proteins: physiological expression in human brain and pathophysiological modulation in multiple sclerosis lesions. *J. Neurovirol.* 11: 23–33.
43. Garson, J., A. Creange, A. Dolei, P. Ferrante, E. Jouvin-Marche, P. N. Marche, F. Rieger, K. Ruprecht, M. Saresella, S. Sotgiu, et al. 2005. MSRV, Syncytin and the role of endogenous retroviral proteins in demyelination. *Mult. Scler.* 11: 249–250.
44. Kohm, A. P., P. A. Carpentier, H. A. Anger, and S. D. Miller. 2002. Cutting edge: CD4⁺CD25⁺ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J. Immunol.* 169: 4712–4716.
45. Pasare, C., and R. Medzhitov. 2003. Toll pathway-dependent blockade of CD4⁺CD25⁺ T cell-mediated suppression by dendritic cells. *Science* 299: 1033–1036.
46. Plumb, J., M. A. Armstrong, M. Duddy, M. Mirakhor, and S. McQuaid. 2003. CD83-positive dendritic cells are present in occasional perivascular cuffs in multiple sclerosis lesions. *Mult. Scler.* 9: 142–147.
47. Huang, Y. M., B. G. Xiao, V. Ozenci, M. Kouwenhoven, N. Teleshova, S. Fredrikson, and H. Link. 1999. Multiple sclerosis is associated with high levels of circulating dendritic cells secreting pro-inflammatory cytokines. *J. Neuroimmunol.* 99: 82–90.
48. Charlton, B., and K. J. Lafferty. 1995. The Th1/Th2 balance in autoimmunity. *Curr. Opin. Immunol.* 7: 793–798.