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Human Rhinoviruses Inhibit the Accessory Function of Dendritic Cells by Inducing Sialoadhesin and B7-H1 Expression

Stefanie Kirchberger,* Otto Majdic,* Peter Steinberger,* Stefan Blüml,* Katharina Pfistershammer,* Gerhard Zlabinger,* Luiza Deszcz,† Ernst Kuechler,2† Walter Knapp,2‡ and Johannes Stöckl3*

Dendritic cells (DC) are professional APCs with an unmatched ability to interact with and activate T cells. There is accumulating evidence that DC not only efficiently stimulate T cell activation but also regulate T cell responses. However, little is known about cell surface structures on DC involved in the regulation of T cell responses. We demonstrate that human rhinoviruses (HRV) can efficiently inhibit the accessory function of DC through induction of inhibitory cell surface receptors. We observed that treatment of DC with HRV14 (R-DC), a member of the major group HRV family, diminished their T cell stimulatory capacity and induced a promiscuous and deep anergic state in cocultured T cells despite high levels of MHC molecules as well as costimulatory molecules, e.g., B7-1 (CD80) and B7-2 (CD86), and independent of inhibitory soluble factors such as IL-10. In contrast, expression of inhibitory B7-H1 molecules was up-regulated and R-DC de novo expressed sialoadhesin (Sn). Most importantly, blocking of B7-H1 and Sn on R-DC with specific mAbs against both receptors reverted the inhibitory phenotype. Thus, inhibitory signals delivered from R-DC to T cells via B7-H1 and Sn were critical for the induction of anergy. These observations suggest that an altered accessory molecule repertoire on DC upon interaction with HRV down-modulates adaptive immune responses during the viral infection. The Journal of Immunology, 2005, 175: 1145–1152.

As the most potent of the professional APCs, dendritic cells (DC) play a central role in the generation of primary T cell responses and the maintenance of immunity (1, 2). Because of their importance in initiating antiviral immune responses, DC represent an ideal target for viral immune-evasion strategies. Several viruses are known to modulate DC function and thus impair antiviral T cell responses (3–5). The result can be a transient or prolonged suppression of the immune response, often associated with secondary microbial infections or the initiation of a persistent infection, both of which represent serious medical problems. However, the mechanism by which viruses induce dysfunction in DC is not fully understood.

The common cold caused by human rhinovirus (HRV) is one of the most frequent infections worldwide (6). HRV primarily infect the ciliated epithelial cells of the nose. Histological examinations of HRV-infected nasal epithelium demonstrated no obvious changes in the morphology or integrity of the nasal epithelium. Instead of cytopathic effects, HRV infection is accompanied with a release of inflammatory mediators (7–9). Attracted by these mediators, inflammatory leukocytes, including DC precursors, infiltrate the mucosal tissue (10, 11). However, despite recruitment of leukocytes, appropriate immune responses appear to be hindered or dysregulated in the respiratory tract upon HRV infection (12). This is implicated by a well-documented 1 predisposition to bacterial infections leading to sinusitis, otitis media, bronchitis, and pneumonia as well as asthmatic exacerbations and 2) delayed HRV-specific immune response (13, 14).

This prompted us to investigate whether HRV might blunt immune responses by modulation of DC function. The results of this study demonstrate that HRV significantly down-regulates the T cell stimulatory capacity of DC through the induction of a novel DC activation program. We could demonstrate that cocultivation of DC with HRV14 (R-DC) induces the expression of inhibitory receptors B7-H1 (PD-1L) and sialoadhesin (Sn, Siglec-1, CD169) without affecting stimulatory receptors such as CD80 or CD86. The consequence of this altered accessory repertoire on R-DC is that cocultured T cells acquire a deep anergic state, which is Ag-nonspecific and not reversible by exogenous IL-2.

Materials and Methods

Media, reagents, and chemicals

Cells were maintained in RPMI 1640 (Invitrogen Life Technologies), supplemented with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (Sigma-Aldrich). Recombinant human GM-CSF, IL-2, and IL-4 were kindly provided by Novartis Research Institute (Vienna, Austria). The HRV-blocking reagent WIN 52035-2 (15) was a kind gift from the Sterling-Winthrop Imaging Research Institute (Rensselaer, NY) and was used at a final concentration of 5 μg/ml. Tetanus toxoid (TT) and purified protein derivative (PPD) of Mycobacterium tuberculosis were purchased from Pasteur Merieux Connaught and used at a concentration of 1 μg/ml. LPS from Escherichia coli (serotype O127:B8), PMA, ionomycin, and propidium iodide were obtained from Sigma-Aldrich. Annexin V...
was provided by Caltag Laboratories. IL-10 was purchased from R&D Systems. Calcein-AM (Molecular Probes) was used at a final concentration of 500 ng/ml.

Ab agents

Monoclonal Abs 7-239 (IgG1) and 7-510 (IgG1) directed against Sn were generated in our laboratory by immunizing BALB/c mice with monocyte-derived DC treated for 1 day with HRV14. Fab of 7-239 were prepared using papain and the recommended protocol from Avidchrom Fab kit (Uni-Syn). Purification was done by affinity chromatography with protein A and size fractionation with fast protein liquid chromatography superdex gel filtration (Pharmacia Biotech). Purity of Fab was checked and confirmed with Abs specific for murine Fab (Sigma-Aldrich) or murine IgG1 (Caltag Laboratories), respectively. All reagents and media used in this study contained <10 pg of LPS/ml.

The following murine mAbs were also generated in our laboratory: negative control Ab VIAP (cell-invasive alkaline phosphatase specific), DF272 (B7-H1), M80 (CD141), VITb6 (CD1a), VIM12 (CD11b), VIM3 (CD14), 4D3 (CD33), 1/47 (MHC class II), 7-480 (CD80), and 7-517 (B7-H3). The CD14 mAb (MEM18) and the CD3 mAb (UCHT-1) were kindly provided by An der Grub (Bio Forschung), and the CD19 mAb (HD37) was a gift from G. Moldenauer (Department of Molecular Immunology, German Cancer Research Center, Heidelberg, Germany). The mAbs DAL-1 (CD80), HB15 (CD83), BI63 (CD86), and 3G8 (CD16) were purchased from Caltag Laboratories. Hybridomas producing mAb W6/32 (MHC class I), G28-5 (CD40), and TS2/52 (CD25) were obtained from American Type Culture Collection (ATCC). mAb RR1/1 (CD54) was a gift from Bender MedSystems. mAb FN50 (CD69) was obtained from BD Pharmingen. The neutralizing polyclonal anti-IL-10 Ab (PAL-IL10) was obtained from Boehringer Mannheim.

Rhinovirus preparation and purification

HRV14 and HRV2 were obtained from ATCC and routinely grown in suspension cultures of HeLa cells (strains Ohio; Flow Laboratories). Preparation and purification of rhinoviruses were performed as described elsewhere (12). Working dilutions of used HRV14 and HRV2 stock preparations contained <10 pg LPS/ml. UV-inactivated virus was prepared by irradiating virus suspensions in a 24-well tissue culture dish (200 µl/well) on ice for 10 min with a 75 W UV source (254 nm) at a distance of 5 cm. Treatment resulted in complete loss of infectious titer. Heat inactivation of virus was performed at 56°C for 1 h.

Generation of monocyte-derived DC

PBMCs were isolated from heparinized whole blood of normal healthy donors by standard density gradient centrifugation with LymphoPrep (Nyegaard). Subsequently, T cells and monocytes were separated by magnetic sorting using the MACS technique (Miltenyi Biotec) as previously described (18). Five rounds of magnetic cell sorting were performed. From the cell pool obtained, single-cell clones were established by limiting dilution culturing.

Assessment of apoptosis and cytokine production

DC (1 × 10⁶/ml) were incubated with HRV14 (1 TCID₅₀ per cell) or LPS (1 µg/ml) in 24-well plates at 37°C for 8 h. Afterward, apoptosis was assessed by staining with FITC-labeled annexin V and propidium iodide and flow cytometric analysis. Annexin V-positive and propidium iodide-negative cells were scored as apoptotic cells. Supernatants of DC and R-DC were analyzed for production of IL-10, IL-2, IL-4, and TNF by sandwich ELISA using matched-pair Abs (R&D Systems). The lower detection limits were 20 pg/ml. Standards consisted of human recombinant material (R&D Systems). Assays were performed in duplicates. Intracellular detection of cytokines was measured as described recently (12) and analyzed by flow cytometry.

Restimulation of T cells

Autologous T cells (1 × 10⁶) were cocultured with TT-pulsed HRV-treated DC. IL-10-treated DC, or untreated DC (1 × 10⁶). After 6 days, T cells were harvested and restimulated with graded numbers of untreated TT-pulsed DC from the same donor as used in the primary MLR. Proliferation of T cells was measured 4 days later.

Flow cytometric analysis

For membrane staining, cells (5 × 10⁵) were incubated for 30 min at 4°C with unconjugated Abs. After washing twice with PBS, Oregon Green-conjugated goat anti-mouse Ig Abs from Molecular Probes was used as a second-step reagent. Flow cytometric analysis was performed using a FACScalibur flow cytometer (BD Biosciences).

Retroviral cDNA expression library

A retroviral cDNA expression library was constructed using mRNA derived from immature, mature, and HRV14-treated DC as a source (1.2 × 10⁸ each). Retroviral vector pBMN (50 µg) (17) containing the cDNA library was introduced into the ecotropic packaging cells (Phoenix-E) by transfection using Lipofectamine Plus reagent (Invitrogen Life Technologies). On days 1, 2, and 3 posttransfection, supernatant was harvested. Fifty milliliters of the filtered (0.45-μm pore size filter) supernatant was supplemented with 50 µl of polybrene (4 mg/ml), added to 1 × 10⁷ target cells in 60 ml of RPMI 1640 containing 10% FCS. Two days after the last infection cycle the cells were used for the selection procedure.

Isolation of mAb 7-239 Ag-expressing cells from a retroviral cDNA library expressing cell pool

The retrovirus-infected cell pool (1 × 10⁶) was used for the selection procedure with MACS (Miltenyi Biotec) and mAb 7-239 (10 µg/ml) as previously described (18). Five rounds of magnetic cell sorting were performed. From the cell pool obtained, single-cell clones were established by limiting dilution culturing.

Molecular cloning of the Sn cDNA insert

Genomic DNA was prepared from a 7-239-reactive, single-cell clone using TRI Reagent (Sigma-Aldrich) following the manufacturer’s instructions. The retrovirus-encoded cDNA inserts were PCR amplified from genomic DNA with the oligonucleotide primers Ban1b (5'-GAGCATCCTCTAATC-3') and Ban2b (5'-GACTGCCGGATC-3') and Ban2b (5'-CATTCCCCCCTTTTCTGAGACTAAAAAAATC-3'), specific for the flanking retroviral sequences. The Long PCR system (MBI Fermentas) was used for the PCR amplification under standard conditions. The obtained PCR products were gelfiltered and cloned using Topo cloning (Invitrogen Life Technologies). Selected plasmids were transfected into 293T cells using Lipofectamine according to the manufacturer’s instructions to confirm that the transfected cells react with the mAb 7-239. Plasmid DNA prepared from selected clones was used for sequence analysis (VBC-Genomics).

Western blotting

Western blotting was performed under standard conditions using mAbs at 1 µg/ml. Bound mAbs were detected using HRP-conjugated goat Abs to mouse Ig (1/10,000; DAKO) and chemiluminescence detection (SuperSignal; Pierce).

Erythrocyte rosetting assay

Freshly isolated human erythrocytes from healthy donors were washed first with RPMI 1650, and mixed with calcium-AM-stained Sn- or control-transduced BsW5147 cells. For inhibition of erythrocyte-rosetting, Ab DF272, 7-239, or Fab 7-239 was added. Transduced cells and erythrocytes were coelectrophoresed at 100 × g for 10 min without brake and further incubated.
at 4°C for 3 h. Rosette formation was investigated using light and fluorescence microscopy. Bw5147 cells with >3 adhered erythrocytes were counted as rosettes.

Results

HRV14 down-regulates the T cell stimulatory function of DC

To analyze the influence of HRV on DC function, we investigated the effect of HRV on DC-induced T cell proliferation. Results presented in Fig. 1A demonstrate that coculture of DC with the major group rhinovirus, HRV14, which uses ICAM-1 as cellular receptor (19, 20), strongly inhibits the T cell stimulatory capacity of DC. No such effect was observed with HRV2, a minor group rhinovirus, which binds to low density lipoprotein (LDL) or LDL-related receptors (21). These effects were observed with purified HRV14 as well as with noninfectious UV-inactivated HRV14, which still binds to ICAM-1 (Fig. 1B). In contrast, DC cocultured with heat-inactivated HRV14 induced the same amount of T cell proliferation as untreated DC (Fig. 1B). Pretreatment of HRV14 with WIN 52032, which sterically blocks the binding sites on HRV for its cellular receptor ICAM-1 (15), prohibited the inhibitory effect of HRV14 (Fig. 1B), indicating that binding of HRV14 to cells was critical.

The down-modulation of the T cell stimulatory function was only seen when DC were cocultured with a minimum of 0.1 multiplicity of infection of HRV14 per cell for at least 24 h (Fig. 1C). The decreased T cell stimulatory capacity was not due to cytotoxic effects of the virus on DC, which are not productively infected by HRV (data not shown). The strongly diminished stimulatory capacity of R-DC was also detectable in autologous MLR and Ag-specific T cell responses to TT and affected CD4+ as well as CD8+ T cell responses (data not shown). Because we have recently shown that HRV14 induces IL-10 production in monocytes we wondered whether IL-10 might be involved (12). However, R-DC generated in the presence of neutralizing IL-10 Abs also showed this strongly reduced T cell stimulatory property, which was even more pronounced than that of IL-10-treated DC (Fig. 1D). Thus, upon binding of HRV14 to DC and coculture for at least 24 h, DC seemingly lose their stimulatory capacity via an IL-10-independent mechanism.

R-DC induce an hypoproliferative state in cocultured T cells

It is well established that IL-10-treated DC are not only poor stimulators of T cells but are capable of inducing Ag-specific anergy in T cells (22, 23). This anergic state cannot be overcome when T cells are restimulated with untreated DC. To test whether R-DC can also induce anergy, autologous T cells were stimulated first with either R-DC, IL-10/DC, or untreated DC pulsed with TT and subsequently restimulated with untreated, TT-pulsed DC.

We observed that T cells stimulated in the primary response with R-DC and IL-10/DC showed reduced proliferation compared with restimulation of T cells primed with untreated DC (Fig. 2A). However, in contrast to IL-10/DC induced anergy, the hypoproliferative response in T cells primed with R-DC was not reverted by addition of IL-2 (Fig. 2B). Moreover and in contrast to IL-10/DC, this anergic state was not Ag-specific because T cells stimulated with R-DC also failed to proliferate upon stimulation with PPD (Fig. 2C).

The reduced proliferative capacity of T cells stimulated with R-DC seems not to be due to increased cell death in T cells as analyzed by annexin V and propidium iodide staining (data not shown). Rather by contrast, T cells cocultured with R-DC show typical blast formation like T cells stimulated with DC and can be induced to express activation markers such as CD69 (Fig. 2D). Moreover, T cells stimulated with R-DC display a cytokine profile (IL-2, IL-4, IL-10, IFN-γ) like T cells stimulated with untreated DC (Fig. 2, E and F). Thus, T cells cocultured with R-DC gain a deep, promiscuous, hypoproliferative state, which is not reversed by exogenous IL-2.

The repertoire of accessory molecules is altered on R-DC

The strongly reduced T cell stimulatory capacity of R-DC prompted us to investigate the molecular basis for this effect. First we analyzed whether soluble factors released by R-DC could account for this inhibitory effect. Results shown in Fig. 3A demonstrate, however, that the supernatant of R-DC like the supernatant of untreated, immature DC when added to an MLR had no inhibitory effect on DC-induced T cell proliferation. Interestingly, when increasing amounts of R-DC were added to DC-T cell cultures we observed that R-DC act inhibitory also under these experimental conditions, again better than IL-10-treated DC (Fig. 3B). Thus, R-DC have a bystander inhibition capacity, which is seemingly located at the cell surface.

Therefore, we next examined the expression profile of cell surface receptors on R-DC in comparison with untreated DC. As shown in Fig. 4A, R-DC express typical DC markers such as CD1a as well as high levels of MHC class I and class II molecules. R-DC do not express typical maturation marker of DC such as CD83. In addition expression of prominent costimulatory molecules, such as B7 family members like B7-1 (CD80) and B7-2 (CD86), B7-H3 (data not shown) was not altered on R-DC. Interestingly, B7-H1

![FIGURE 1](http://www.jimmunol.org/)

Coculture of DC with HRV14 inhibits their T cell stimulatory capacity. A, Purified T cells were stimulated with graded numbers of allogeneic DC inoculated with HRV14 (△), HRV2 (□) for 24 h, or mock-treated DC (○). Proliferation of T cells was monitored on day 5 of culture by adding [methyl-3H]TdR followed by measuring [methyl-3H]TdR incorporation 18 h later. The results of one representative experiment of four independent experiments are shown. B, DC were treated for 24 h with purified HRV14 (△), heat-inactivated HRV14 (○), UV-inactivated HRV14 (□), or HRV14 pretreated with WIN 52032 (5 μg/ml) (□) or mock-treated (○) and used as stimulators for T cell proliferation. T cell proliferation was measured as described in A. The results are representative of two independent experiments. C, DC were cocultured with HRV14 for 1 h (□) or 24 h (△) or mock-treated (○) and used as stimulators for allologenic T cells as described in A. The results are representative of three independent experiments. D, DC were treated with HRV14 (triangles) or IL-10 (squares) in the absence (filled symbols) or presence (open symbols) of neutralizing IL-10 Abs and mock (○) and used as stimulators for allogenic T cells as described in A.
(PD-L1), an inhibitory receptor on DC involved in the induction of T cell anergy by IL-10/DC (24), was up-regulated on R-DC.

To analyze whether enhanced expression of B7-H1 on R-DC might account for the inhibitory effect, we blocked B7-H1 on R-DC with mAb DF272, a procedure that we have recently found to restore the stimulatory capacity of IL-10/DC. However, as shown in Fig. 4B, mAb DF272 only weakly improved the stimulatory capacity of R-DC. Thus, additional receptors are obviously involved in the inhibitory capacity of R-DC.

Search for cell surface receptors selectively expressed on R-DC

To identify candidate receptor structures on R-DC, which might be responsible for the inhibitory phenotype, we immunized mice with R-DC, generated mAbs and screened for mAbs, which preferentially react with R-DC but not with untreated DC. One Ab, which fulfilled these criteria, was mAb 7-239. As shown in Fig. 5A, mAb 7-239 showed weak reactivity with untreated DC but strong reactivity with DC treated with HRV14 for 24 h. Interestingly, the 7-239-defined molecule was not induced on DC with HRV2. mAb 7-239 recognizes a 210-kDa cell surface protein (Fig. 5B). To identify the molecule recognized by the mAb 7-239, an expression cloning approach was used (18, 25, 26). A retroviral cDNA library constructed from human monocyte-derived DC and R-DC was expressed in mouse target cells. The transduced cell pool was subjected to five rounds of selection with mAb 7-239 (Fig. 5C). The PCR product obtained from a single-cell clone was introduced into a eukaryotic expression vector (Fig. 5D). Cells expressing the protein encoded by the 7-kb PCR product were specifically recognized by mAb 7-239 (Fig. 5E). The cDNA insert was sequenced and was found to be identical with Sn (27–29).

Sn is a member of the sialic acid binding lectin family, which has a carbohydrate-binding domain at the N terminus. A characteristic feature of Sn is that it mediates binding of erythrocytes (28). Interestingly, mAb 7-239 was found to completely inhibit erythrocyte rosetting around Sn+/H11001 transductants (Fig. 5F), indicating that mAb 7-239 binds to or close to the lectin domain of Sn.

Blocking of B7-H1 and Sn restores the stimulatory function of R-DC

Sn is known as an adhesion molecule and a macrophage-specific marker (29). Our finding that Sn is induced on DC upon HRV14 cocultivation raised the question of whether Sn might act as an inhibitory receptor on R-DC for T cells. Results presented in Fig. 6 demonstrate that engagement of Sn with mAb 7-239 on R-DC enhanced proliferation of cocultured T cells. Similar to the B7-H1 mAb DF272 the Sn mAb 7-239 was not able to fully revert the inhibitory effects of rhinovirus treatment on DC (Fig. 4B). However, when we added mAb 7-239 and mAb DF272 in combination, R-DC regained a T cell stimulatory capacity like untreated DC. Addition of mAb 7-239 or DF272 in combination with mAbs
against other DC cell surface receptors such as mAb M80 (CD141) showed no such effect (Fig. 6, A–C). R-DC treated with mAb 7-239 or mAb DF272 did not show increased expression of activation marker (CD80, CD83, CD86, or HLA-DR) or cytokine production (IL-1, TNF-α, IL-12, or IL-10), typical signs for activated DC (data not shown). Thus, induction of DC activation upon binding of mAbs 7-239 and DF272 does not seem to be responsible for this enhanced accessory function. Most importantly, like with intact Abs, treatment of R-DC with Fab of mAbs 7-239 and DF272 enhanced their stimulatory function (data not shown). Thus, binding of mAbs DF272 and 7-239 to B7-H1 and Sn on R-DC, respectively, seemingly prevents the delivery of inhibitory signals to T cells leading to anergy induction.

Discussion
Viruses have established numerous strategies to survive and to evade antimicrobial strategies of the host (3). They can interfere at several steps of DC-induced immunity including generation, survival, migration, and maturation of DC, processing/presentation of Ags by DC and activation of T cell responses by DC. A particularly efficient trick of viruses, e.g., vaccinia virus, measles, HIV, or CMV, is the targeting of the costimulatory function of DC. Thereby the virus is able to prevent pathogen-specific immunity (i.e., ignorance) and, even more effective, is able to induce pathogen-specific tolerance, when DC present Ags in the absence of costimulatory signals (22, 23). In this study, we demonstrate that up-regulation of inhibitory accessory molecules, i.e., B7-H1 and Sn, on DC represents another efficient viral strategy to turn off Ag-specific immune responses.

We discovered this novel mechanism of virus-induced immunosuppression by investigating the impact of HRV14, a member of the major group HRV family, on DC function. Inhibition of Ag-specific T cell responses by major group HRV have been reported previously by our group and others using monocytes as APC (12). We could demonstrate that these inhibitory effects resulted from a
particular spectrum of cytokines released by monocytes upon HRV14 interaction. Most prominent among them is IL-10, a well-established immunosuppressive cytokine, which was strongly induced by HRV14 (12). Because monocytes are the precursor cells of our DC, we expected that IL-10 production induced upon coculture of DC with HRV14 was involved in this process. Yet, although small amounts of IL-10 are produced by R-DC, neutralizing Abs against IL-10 failed to reverse the inhibitory effect of HRV14. In addition, transfer experiments with the supernatant of virus to the MLR cultures or due to a brief pretreatment of DC with HRV14 but required a coculture of at least 24 h.

During this time, HRV14 obviously modulates the costimulatory repertoire of DC in a special way. The expression of most costimulatory molecules analyzed in this study was not affected on R-DC. For instance, CD40, CD58, CD80, and CD86 expression was similar on R-DC and on untreated DC. Likewise, expression of MHC class I and class II molecules, a known target of viral escape mechanisms (4, 5), was not down-regulated on R-DC. Thus, the clearly reduced stimulatory capacity of R-DC did not correlate with a reduced ability to deliver signal 1 and 2 to T cells. Because we observed that R-DC gain a bystander inhibitory function we concluded that HRV14 may alter the accessory phenotype by inducing inhibitory receptors on DC.

We could indeed identify two such receptors on R-DC, which seem to deliver inhibitory signals to T cells, B7-H1 and Sn.

A retroviral cDNA library constructed from human monocyte-derived DC was expressed in mouse target cells. The transduced cell pool was subjected to five rounds (a, round 1; b, round 3; and c, round 5) of selection with mAb 7-239. D. Genomic DNA derived from a 7-239 reactive single cell clone was PCR-amplified using primers specific for the retroviral cDNA inserts. E. Cells expressing the protein encoded by the 7-kb PCR product were specifically recognized by mAb 7-239. F. A characteristic feature of Sn is that it mediates binding of erythrocytes. Addition of mAb 7-239 inhibits erythrocyte rosetting around Sn+ transductants indicating that mAb 7-239 binds to or close to the lectin domain of Sn. Mean values ± SD of three experiments are shown.
not expressed on monocytes, lymphocytes and DC (27). Our finding that Sn is strongly induced on DC upon HRV14 treatment after 1–2 days correlates with the appearance of the inhibitory phenotype of R-DC. Interestingly, IL-10 did not induce expression of Sn on DC (data not shown). CD43 has been recently described as ligand for Sn on T cells (32). How might this interaction contribute to the inhibition of T cell activation by R-DC? CD43 is an active process dependent on reorganizations of the cytoskeleton. If an APC like our R-DC expresses a counterreceptor for CD43, it seems to be intriguing that this interaction might interfere with an appropriate movement of CD43 to the distal pole. As a consequence, synapse formation required for complete T cell activation might be disturbed. Together with inhibitory signals arising from B7-H1–PD-1 interactions this might explain the induction of a deep anergy in T cells upon R-DC activation. We are currently investigating whether Sn-CD43 interaction indeed affect synapse formation in T cells.

The mechanism of B7-H1 and Sn induction in R-DC and the role of ICAM-1 are not yet understood. Signal transduction via ICAM-1 has been described. It can be induced by specific Abs (38, 39) or by its ligand fibronogen (39). We observed that engagement and cross-linking of ICAM-1 with CD54 mAb RR1/1 did not trigger Sn expression on DC but inhibited HRV-induced Sn expression (data not shown). However, because one HRV capsid has 60 receptor binding sites and binds to ICAM-1 with high avidity (40, 41), HRV particles might trigger pronounced cross-linking of ICAM-1 and subsequent signal transduction leading to Sn induction and up-regulation of B7-H1 expression. It is conceivable that this may not be achieved with Abs. Interestingly, ICAM-1 is not only the receptor for rhinoviruses but also for other pathogens including coxsackie viruses and Plasmodium falciparum. For Plasmodium-infected erythrocytes it indeed has been recently demonstrated that they inhibit DC maturation (42). Thus, ICAM-1 is not only an important adhesion molecule but like TLRs may also play an important role in sensing pathogens. However our observations implicate that in contrast to TLRs, engagement of ICAM-1 on DC might trigger inhibitory signals that shut off the stimulatory function of DC.

On the basis of these observations it is tempting to speculate that the unique modulation of DC function by HRV might have adverse effects on local immunity in areas of HRV infection. Such reduced local immunocompetence may predispose affected individuals to secondary infections and could explain the frequently observed occurrence of sinussitis, otitis media, bronchitis, and pneumonia in HRV-infected individuals.

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Disclosures
The authors have no financial conflict of interest.

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