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The Poliovirus Receptor CD155 Mediates Cell-to-Matrix Contacts by Specifically Binding to Vitronectin

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The human receptor for poliovirus (CD155) is an immunoglobulin-like molecule with unknown normal function(s). Here we provide evidence that CD155 binds specifically to vitronectin with a dissociation constant (K_d) of 72 nM as determined by surface plasmon resonance. Based on sequence homology to the *CD155* gene, three poliovirus receptor-related genes (*PRR1, PRR2,* and *PRR3*) were cloned recently. PRR proteins were reported by others to mediate homophilic cell adhesion. Neither PRR1 nor PRR2 binds poliovirus and it is assumed that their physiological functions differ from that of CD155. Indeed, mPRR2 was found to bind to vitronectin only weakly, while its self-adhesion activity is characterized by a K_d of 310 nM. Moreover, there is no evidence for CD155 self-adhesion. Both CD155 and vitronectin colocalize to follicular dendritic cells and B cells inside the germinal centers of secondary lymphoid tissue (tonsils)—an observation suggesting that the CD155/vitronectin interaction is required for the establishment of a proper immune response in this particular context.
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Key Words: poliovirus receptor; CD155; poliovirus receptor related; nectin; vitronectin; germinal center—follicular dendritic cell.

INTRODUCTION

CD155 is a single-pass transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily of proteins. The extracellular portion of CD155 is composed of three Ig-like domains: an outermost V-like domain followed by two C2-like domains (Fig. 1; Wimmer *et al.,* 1994). CD155 was originally discovered as the cellular receptor for poliovirus (PV) (PVR; Koike *et al.,* 1990; Mendelsohn *et al.,* 1989) with the binding occurring between the V-like domain and the virion (Wimmer *et al.,* 1994). Although earlier studies indicated that CD155 is widely distributed throughout human tissues, its level of expression is low and stringently controlled (Freistadt, 1994; Solecki *et al.,* 1997, 1999, 2000). Due to alternative splicing, the gene can give rise to four different receptor proteins: two membrane-bound receptors (CD155α and CD155δ) and two potentially secreted isoforms (CD155β and CD155γ) which lack the transmembrane region (Bernhardt *et al.,* 1994a; Bibb *et al.,* 1994a,b; Koike *et al.,* 1990). Whereas the interaction between PV and its receptor was studied in great detail (Belnap *et al.,* 2000; He *et al.,* 2000; Wimmer *et al.,* 1994), the physiological function(s) of CD155 remained obscure. CD155 is the founding member of a growing family of related molecules sharing the V-C2-C2 architecture. Tage4 was identified as a tumor antigen overexpressed in rat colon carcinomas (Chadeneau *et al.,* 1994; Denis, 1998). In addition, three human poliovirus receptor-related cDNAs (hPRR1, hPRR2, and hPRR3) were identified (Eberle *et al.,* 1995; Lopez *et al.,* 1995; Reymond *et al.,* 2000). Sequence comparisons revealed that hPRR2 is the human counterpart of mPRR2, originally termed MPH (Morrison and Racaniello, 1992; murine pvr homolog, see Fig. 1 for the extracellular domains). The latter was assumed mistakenly to represent the murine homolog of CD155. Thus, homologs of CD155 could be identified only in monkeys (Aoki *et al.,* 1994; Koike *et al.,* 1992), suggesting that these receptors are unique to primates. Based on recent evidence, Baury *et al.* hypothesized that rat Tage4 is the rodent homolog of CD155 (Baury *et al.,* 2001). However, sequence features and chromosomal locations rather indicate the existence of two subfamilies: CD155, Tage4, and PRR2 may be grouped into a PVR subfamily, whereas PRR1 and PRR3 represent the PRR subfamily. Neither PRR1 nor PRR2 mediates entry of PV into susceptible cells yet they serve as receptors for different species of α-herpesviruses (Cocchi *et al.,* 2000; Geraghty *et al.,* 1998; Shukla *et al.,* 1999). Apart from this pathogenic function, PRRs mediate homophilic cell adhesion and were shown to couple via afadin, an actin filament binding protein, to the E-cadherin system (Aoki *et al.,* 1997; Lopez *et al.,* 1998;

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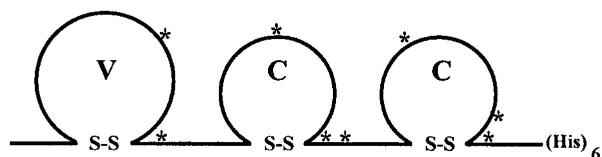

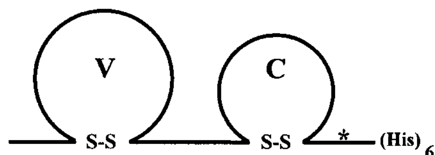

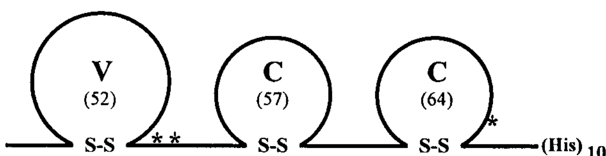

FIG. 1. Schematic representation of the recombinant receptors sCD155d1d3, sCD155d1d2aglc, and smPRR2d1d3. Circles denote the Ig-like domains: V for variable, C for constant. Receptors were tagged with a His epitope. Asterisks indicate N-linked glycosylation sites. Numbers in parentheses (smPRR2) indicate the degree of sequence homology in percentage between the Ig-like domains of CD155 and mPRR2.

Miyahara *et al.,* 2000; Tachibana *et al.,* 2000; Takahashi *et al.,* 1999). Mice deficient in mPRR2 expression were found to be sterile due to a defect in spermatogenesis (Bouchard *et al.,* 2000).

Vitronectin is an important component of the extracellular matrix and constitutes a major serum protein. It is expressed abundantly by liver cells and secreted into the blood stream (see Preissner, 1991, for a review). Vitronectin was shown to bind several cell receptors predominantly of the integrin type as well as various serum factors. Therefore, vitronectin is implicated in diverse processes such as cell attachment and migration, thrombosis, fibrinolysis, and neoplasia (reviewed by Schvartz *et al.,* 1999). Moreover, vitronectin has been assigned a role in cell differentiation during embryogenesis (Martínez-Moralez *et al.,* 1997). Another important feature relates to the binding of vitronectin to proteins of the complement system—an interaction resulting in the inhibition of the complement cascade (Preissner, 1991). Despite these multiple roles, knockout mice deficient for vitronectin develop normally (Zheng *et al.,* 1995) but show an increased rate of thrombus formation (Fay *et al.,* 1999).

Here we report that CD155 binds specifically to vitronectin. The vitronectin/CD155 interaction is distinguished by a rapid complex formation and a dissociation constant of 72 nM. Furthermore, CD155 and vitronectin are coexpressed on follicular dendritic cells and B cells inside germinal centers of secondary lymphoid organs. The significance of these findings will be discussed.

RESULTS

To investigate the interaction of CD155 with components of the extracellular matrix, adhesion assays were performed using human vitronectin as substrate. HEp2 cells known to express CD155 were found to attach readily to vitronectin-coated plastic wells. Monoclonal antibodies (mAb) D171 and P44, which recognize CD155, were found to reduce binding of the cells to vitronectin significantly, whereas an unrelated isotype control antibody (9E10) exerted no effect (Fig. 2a, closed bars). Since mAbs D171 and P44 completely block the interaction between PV and the V-like domain of CD155 (reviewed by Wimmer *et al.,* 1994), our observations indicate that the V-like domain may also be involved in vitronectin binding. When HEp 2-Cl32 cells lacking CD155 expression were used in the assays, inhibition of binding by D171 or P44 was marginal and most likely resulted from nonspecific interactions (Fig. 2a, open bars). However, it should be noted that even in the presence of saturating amounts of either D171 or P44, or a combination thereof, binding of the HEp 2 cells to the substrate was not completely abolished (data not shown). This observation suggested that a second receptor might be involved in cell/substrate attachment. Therefore, mAb 23C6, known to block the adhesive function of the classical vitronectin receptor $\alpha_v\beta_3$, was used in these binding assays. MAb 23C6 caused a considerable reduction of HEp 2 cell binding (Fig. 2b, closed bars). Similar to the D171 blocking experiments, an increase in the concentration of mAb 23C6 up to 10-fold did not further reduce binding (data not shown). In contrast, a combination of mAbs D171 and 23C6 resulted in almost background levels of HEp 2 cell binding. As anticipated, the attachment of HEp 2-Cl32 was also affected by mAb 23C6, albeit to a slightly lesser degree compared to HEp 2 cells (Fig. 2b, open bars). The combined application of antibodies 23C6 and D171 had no additive effect on HEp 2-Cl32 binding to vitronectin. As expected, the isotype control mAb 9E10 did not reduce binding of HEp 2-Cl32 cells (Fig. 2b).

The bulky antibodies forming a complex with CD155 might have masked vitronectin-binding epitopes that were provided by molecules located in direct proximity to CD155. Indeed, it has been reported that CD155 is physically associated with CD44 on human mononuclear blood cells (Freistadt and Eberle, 1997). In addition, our own analyses confirmed expression of CD44 on HEp 2 cells, as well as on HEp 2-Cl32 cells (data not shown). Therefore, a recombinant protein consisting of the ectodomain of the receptor (sCD155d1d3, Fig. 1) was

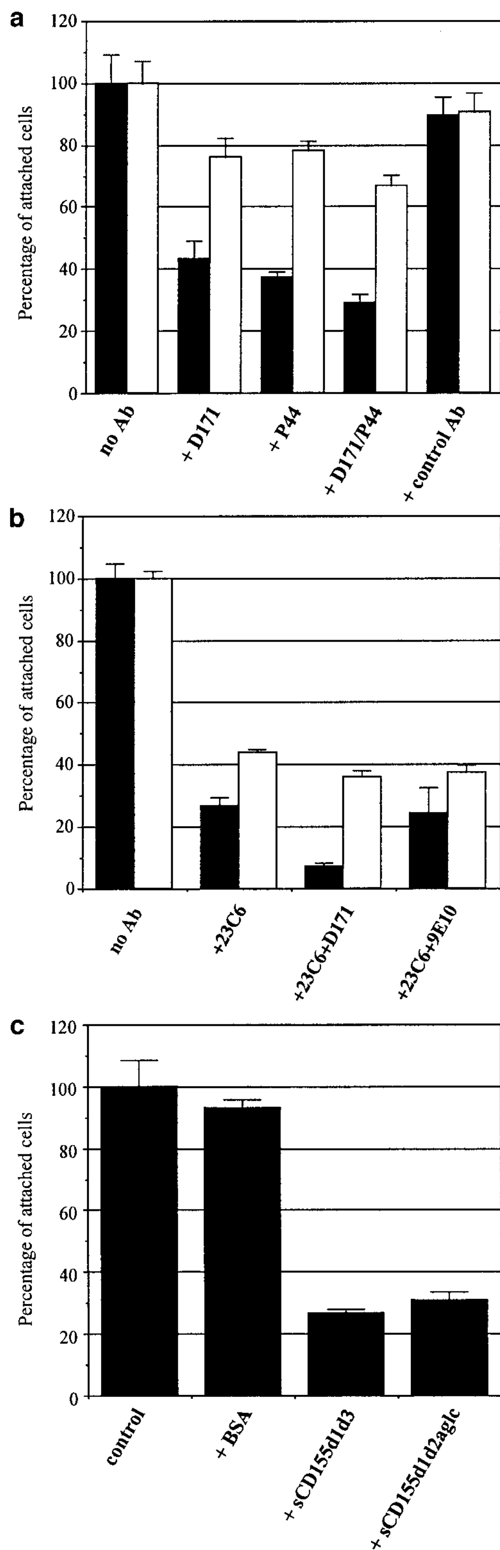

FIG. 2. Binding of HEp 2 and HEp 2-Cl32 cells to nitronectin. Vitronectin was coated onto 96-well plastic dishes (see Materials and Methods for details). (a) Inhibition of binding of HEp 2 (closed bars) and HEp 2-Cl32 cells (open bars) to vitronectin-coated dishes in the absence (100%) or the presence of 50 μg of mAb as indicated. In the case of D171/P44 (both mAbs are directed against CD155) a 1:1 mixture was applied. (b) Inhibition of binding of HEp 2 cells (closed bars) and HEp 2-Cl32 cells (open bars) to vitronectin by 10 μg of mAb 23C6, an a_vb_3 integrin-specific antibody, or by mixtures of 10 μg mAb 23C6 with either 50 μg D171 or 50 μg 9E10, as indicated. (c) Binding of HEp 2 cells (100%) compared to the binding in the presence of 10 μg of BSA, sCD155d1d3, or sCD155d1d2aglc. All assays were done at least three times in duplicate. Shown are the means; SD (standard deviations) are indicated by error bars.

produced and used in competition experiments. The structural integrity of sCD155d1d3 was confirmed by its ability to reduce the titer of PV approximately 500-fold (see Materials and Methods). The recombinant protein sCD155d1d3 was found to decrease binding of HEp 2 cells to vitronectin by 75% relative to the control (Fig. 2c). Under these experimental conditions, the binding of HEp 2-Cl32 cells to the substrate was reduced by approximately 20% (data not shown).

Almost half of the molecular mass of CD155 is contributed by sugar chains (Fig. 1; Bernhardt *et al.,* 1994a). Since vitronectin is known to interact with carbohydrate-derived ligands, it was of interest to determine whether the sugar moiety of CD155 contributes to vitronectin binding. A recombinant protein consisting of the two N-terminal CD155 domains but lacking four of the five putative N-glycosylation sites (Fig. 1, sCD155d1d2aglc) (Bernhardt *et al.,* 1994b), was expressed in CHO cells and purified. Recombinant protein sCD155d1d2aglc interfered with HEp 2 cell binding to vitronectin to an extent comparable to that of sCD155d1d3 (Fig. 2c). These data suggest that the CD155/vitronectin interaction is mediated by an epitope residing in the V-2C domains, most likely the N-terminal V-like domain (see above). Since the V-like domain of recombinant sCD15d1d2aglc is devoid of N-linked glycomodifications, it is conceivable that this interaction is independent of the glycosylation pattern of CD155.

These experiments were followed by dot blot assays applying sCD155d1d3 as a detector (see Materials and Methods). Polypeptide sCD155d1d3 specifically recognized vitronectin but not filter-bound collagen type IV or BSA (Fig. 3). In addition, we observed specific binding of sCD155d1d3 to bovine and rat vitronectin, albeit at slightly reduced levels compared to human vitronectin (data not shown).

The specific interaction between CD155 and vitronectin was then quantitated by surface plasmon resonance (BIAcore). In these experiments the proteins of interest (ligands, see also Tables 1 and 2) are immobilized onto a sensor chip. The putative interaction with another soluble entity (the analyte, here soluble recombinant CD155 or mPRR2) is subsequently monitored under constant flow conditions, varying the concentrations of the analyte under investigation. As a result, on and off rates as well as the dissociation constant (K_d) of a distinct binding reaction can be determined. MAb D171 bound to sCD155d1d3 with a K_d of 8 nM (see Table 1), suggesting that this approach is suitable to study the interaction of

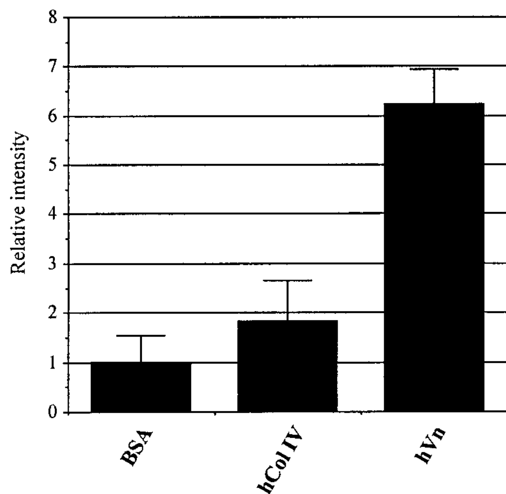

FIG. 3. Binding of sCD155d1d3 to various substrates. Human vitronectin (hVn), human collagen type IV (hColIV), and BSA were spotted onto nitrocellulose. Binding of sCD155d1d3 was monitored as described under Materials and Methods. The intensity reflecting the amount of bound sCD155d1d3 was set to 1 in the case of BSA.

CD155 with putative ligands. sCD155d1d3 bound to human vitronectin with a K_d of 72 nM. In contrast, the K_d values for the interaction of sCD155d1d3 with human collagen type IV and BSA were 46 and 21 μM, respectively. K_d values in the latter range were considered to represent nonspecific binding.

Similar binding experiments were carried out with mPRR2, a mouse receptor related to CD155. For this purpose, the ectodomain of mPRR2 (smPRR2d1d3; see Fig. 1) was expressed in insect cells and purified. Rat vitronectin, displaying a very high degree of conservation compared to mouse vitronectin, was chosen as a substrate (Otter *et al.,* 1995). Polypeptide smPRR2d1d3 bound to rat vitronectin with a K_d of 5 μM. As shown in Table 2, we have confirmed the homophilic interaction between smPRR2d1d3 molecules (K_d of 310 nM). Based on experiments in which mPRR2 was expressed on the surface of insect cells, this property has already been described (Aoki *et al.,* 1997). In similar assays we expressed membrane-bound CD155 in insect cells in order to check for cell clustering. However, such cells failed to aggregate (data not shown), confirming the notion of others that CD155 does not possess homophilic adhesion potential (Aoki *et al.,* 1997).

TABLE 1

Rate Constants for sCD155d1d3

Ligand	K_d (M)	Off rate (s^{-1})	On rate (M^{-1} s^{-1})
D171	8.1×10^{-9}	8.0×10^{-3}	9.8×10^{5}
hVn	7.2×10^{-8}	7.4×10^{-3}	1.0×10^{5}
hColIV	4.6×10^{-5}	5.2×10^{-3}	102
BSA	2.1×10^{-5}	5.1×10^{-3}	234

Note. Shown are the means of at least three experiments with the listed ligands coupled onto the sensor chip (see Materials and Methods). hVn, human vitronectin; hColIV, human collagen type IV.

TABLE 2

Rate Constants for smPRR2d1d3

Ligand	K_d (M)	Off rate (s^{-1})	On rate (M^{-1} s^{-1})
rVn	5.0×10^{-6}	7.7×10^{-4}	140
smPRR2	3.1×10^{-7}	5.8×10^{-5}	186
BSA	1.5×10^{-4}	3.7×10^{-3}	25

Note. Shown are the means of at least three experiments with the listed ligands coupled onto the sensor chip (see Materials and Methods). rVn, rat vitronectin.

We then searched for the CD155/vitronectin interaction *in vivo* and chose to investigate the expression of both molecules in human tonsilar sections by immunohistochemistry. Upon ingestion, the first target of PV are the tonsils (Melnick, 1996), suggesting expression of CD155 in as yet unidentified cell populations inside this secondary lymphoid tissue. Staining with mAb D171 revealed the presence of CD155 within the germinal centers (GC) (see Figs. 4a and 4b). It was already shown earlier that vitronectin is also present in GCs (Halstensen *et al.,* 1988). In order to determine whether its expression overlapped that of CD155, we used a specific antibody to visualize vitronectin (see Figs. 4c and 4d). The colocalization of the two molecules in GCs was evident when CD155 (red) and vitronectin (green) were detected in dual staining experiments (Fig. 4h). GCs represent the sites of a secondary immune response, are relatively short-lived, and possess a dynamic structure. They are composed predominantly of B cells, which undergo clonal expansion, hypermutation, positive or negative selection, and memory B cell generation. These processes are dependent upon the support by interdigitating cells, T cells, and follicular dendritic cells (FDC) (reviewed by Liu *et al.,* 1996). Dual staining experiments would suggest that in addition to the latter cell type (see Fig. 4e), GC B cells express CD155 (see Figs. 5c and 5e). Apparently, GC B cells differ from FDCs in expressing CD155 to various extents (Figs. 5c and 5e). Concomitantly, we observed a similar bias in the vitronectin staining pattern (see Figs. 5d and 5f). In contrast, T cells were found to be devoid of CD155 (Fig. 5a). T cells accumulate in the areas surrounding the GC and only a few T cells are interspersed among the cells comprising the GC. Therefore, it is possible that FDCs and GC B cells bind vitronectin by expressing CD155 on their surface (see colocalization of these molecules in Figs. 4g, 5d, and 5f). This view is challenged by the fact that vitronectin can interact with several other cell receptors, most notably integrins (Preissner, 1991). However, we failed to detect a GC-specific staining using an antibody directed against the human a_v chain (Fig. 5b). Interestingly, the a_v chain is part of almost all vitronectin receptors of the integrin type, corroborating the finding of

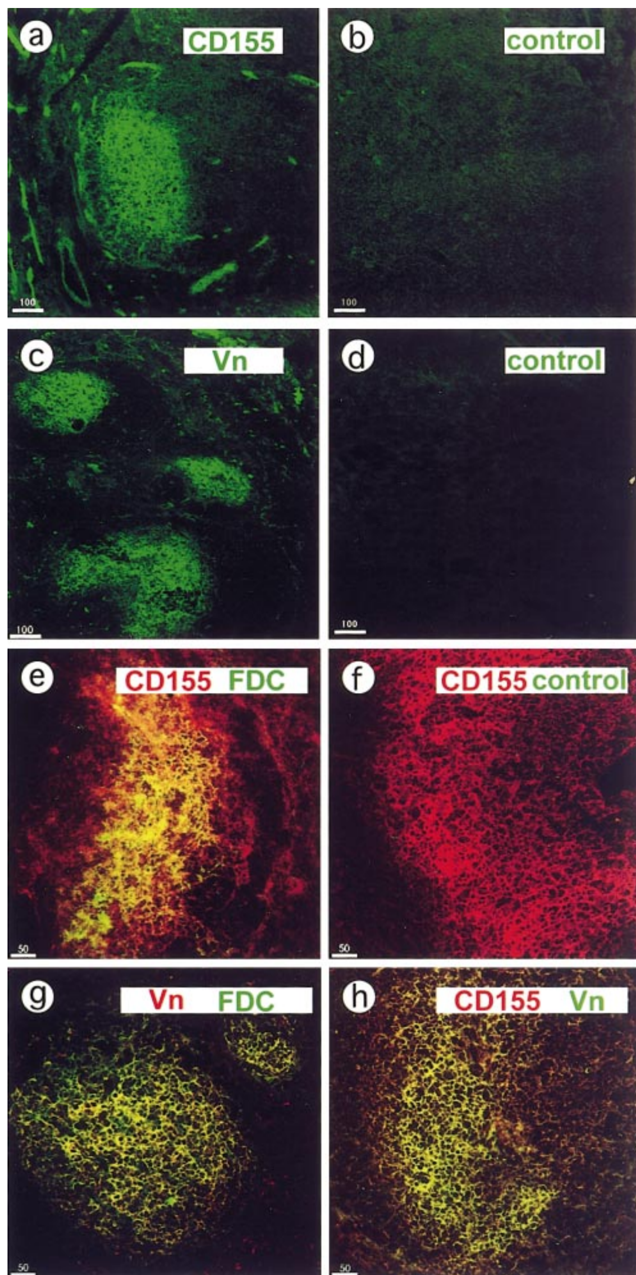

FIG. 4. Colocalization of CD155 and vitronectin on FDCs. Cryosections of human tonsils were stained with mAbs directed against CD155 (D171), vitronectin (clone Vn5-3), and follicular dendritic cells (HJ2), in single (a–d) or double (e–h) staining experiments. The white bars represent the indicated size in micrometers, whereas the molecule detected is depicted in color on the top. Control denotes a cryosection stained with an isotype control antibody for (b) D171, (d) clone Vn5-3, and (f) HJ2.

others that integrin-like vitronectin receptors are absent on cells inside the GCs (Castanos-Velez *et al.,* 1995).

DISCUSSION

The identification of the cellular receptor of poliovirus (CD155) in 1989 led to the discovery of a new family of Ig-like molecules, termed PRRs (poliovirus receptor related; see Introduction). While the normal function(s) of CD155 remained enigmatic, PRRs were shown to serve as homophilic adhesion receptors engaged in the establishment of tight adherens junctions. Therefore, these molecules had been renamed nectins (nectin 1, 2, and 3; Takahashi *et al.,* 1999).

In our search for ligands of CD155, we observed that a cell line (HEp 2) expressing CD155 could bind to vitronectin-coated matrices. We then found that this interaction is mediated not only by CD155 but independently also by an integrin. Corresponding antibodies (Fig. 2) could specifically block both binding events. The binding of HEp 2 cells to vitronectin was almost completely inhibited in the presence of both antibodies (see Fig. 2b). As expected, only the vitronectin-specific antibody inhibited the attachment of cells lacking CD155 (HEp 2-Cl32), whereas mAb D171 had little effect.

Soluble recombinant derivatives of CD155 (sCD155d1d3 and sCD155d1d2aglc, Fig. 1) competed with binding of HEp 2 cells to vitronectin (Fig. 2c), an observation supporting our conclusion of CD155-mediated attachment. Direct evidence for an interaction between vitronectin and sCD155d1d3 was obtained in a filter-based adhesion assay (Fig. 3) and by calculating dissociation constants using surface plasmon resonance experiments (Table 1). sCD155d1d2 bound to human vitronectin with a K_d of 72 nM, a value within the limits of K_d's determined for other vitronectin ligands such as heparin (10 nM) and uPAR (30 nM) (Preissner, 1991; Wei *et al.,* 1994). In contrast, human collagen type IV and BSA displayed characteristics of a nonspecific binding (demonstrated by their low K_d values of 46 and 21 μM, respectively).

Similar experiments performed with a soluble form of mouse PRR2/nectin-2 (smPRR2) revealed a low affinity of mPRR2 to rat vitronectin (K_d of 5 μM, see Table 2). In contrast, the observed homophilic adhesion of smPRR2 is characterized by a K_d of 310 nM, a value comparable to those obtained for other Ig-superfamily proteins such as N-CAM (K_d of 64 nM; Retzler *et al.,* 1996). The homophilic interaction of membrane-associated mPRR2/nectin-2 may gain in strength since it has been speculated that mPRR2/nectin-2 need to dimerize *in cis* in order to bind efficiently *in trans* (Miyahara *et al.,* 2000).

Previous observations indicated that both vitronectin and CD155 are expressed in tissue of human tonsils. Therefore, we studied the distribution of these proteins in this anatomical compartment making use of markers specific for CD155, vitronectin, T cells, GC B cells, and FDCs. In single or combined immunohistological stainings, it was evident that vitronectin (Fig. 4c) and CD155 (Fig. 4a) colocalize inside the GC (Fig. 4h). The presence of CD155 was also apparent on FDCs and GC B cells (Figs. 4e and 5d), where its expression overlapped substantially that of vitronectin. It is remarkable that GCs are

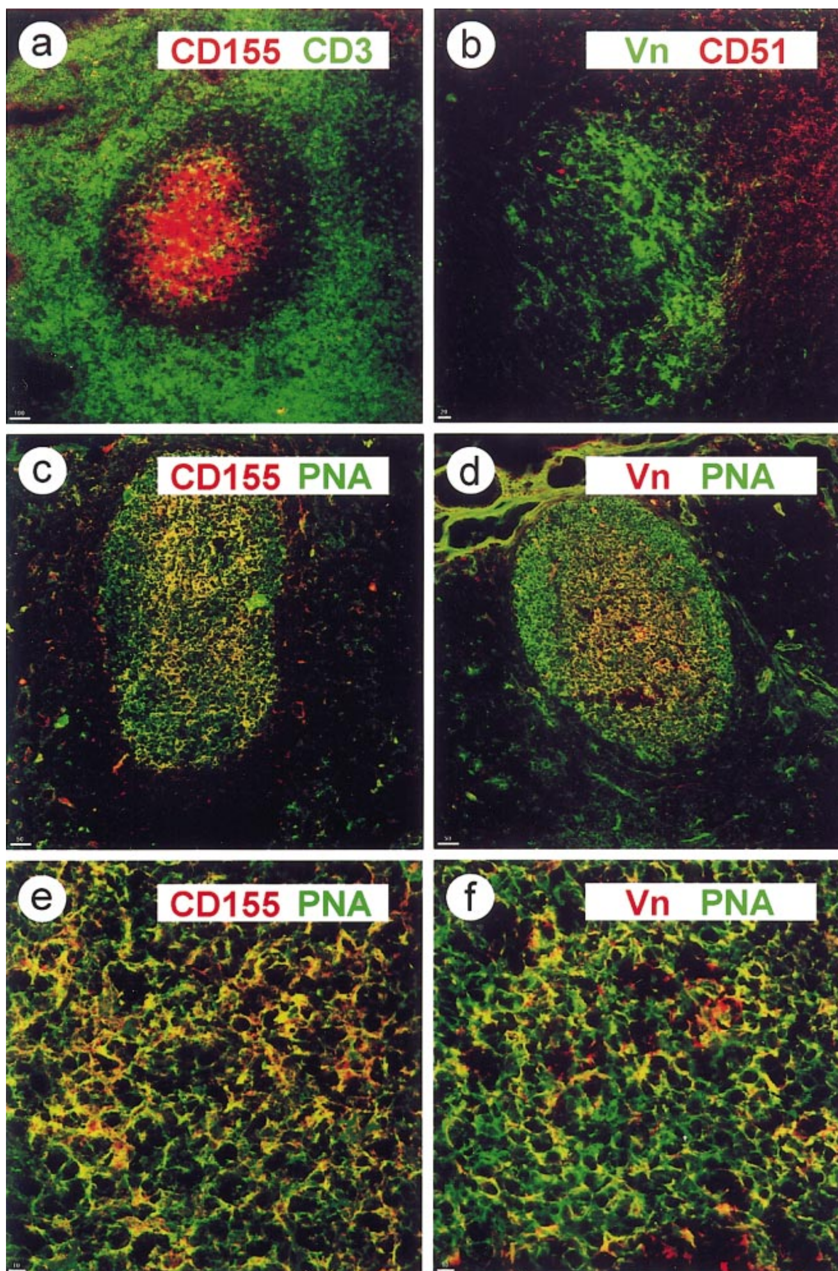

FIG. 5. Colocalization of CD155 and vitronectin on B cells. Human tonsillar cryosections were stained with antibodies specific for CD155 (D171), CD3 (clone UCHT-1), CD51 (a_v integrin subunit-specific antibody, N-19), or vitronectin (clone VN5-3). B cells inside the GC were stained using the lectin PNA. CD155 was not found to colocalize with either T cells (a) or the α_v chain of integrins (b), whereas B cells express CD155 (c) and are decorated with vitronectin (d). (e) A closer view of a GC double stained for CD155 and GC B cells and (f) a corresponding GC stained for vitronectin and GC B cells.

devoid of other important ECM components such as collagens, laminin, fibronectin, and tenascin (Castanos-Velez *et al.,* 1995). Similarly, the α and β chains of many of the integrin receptors of these ECM proteins are also either absent or only weakly expressed in GCs (Castanos-Velez *et al.,* 1995). In particular, those receptors known to interact with vitronectin are missing, a finding corroborated by our observation that an α_v-specific antibody failed to stain specific structures inside the GC (Fig. 5b). FDCs retain on their surface complement protein C3 bound to an antigen that is subsequently presented to B cells (see Carroll, 1998b, for a review). These C3 complexes may be taken up and processed by GC B cells contacting FDCs (Carroll, 1998b). The expression of complement receptors CD21 and CD35 on the surface of both FDCs and GC B cells is a prerequisite for a successful antigen-triggered immune response (Carroll, 1998a; Fang *et al.,* 1998; Fischer *et al.,* 1998). Therefore, proteins of the early complement cascade *and* their receptors are involved in proper GC development as well

as memory B cell generation (Carroll, 1998b; Liu *et al.,* 1996). However, complement can also be harmful in this environment by forming membrane attack complexes deadly to the cells, a fate that can be prevented by vitronectin (Preissner, 1991). Indeed, immunostainings indicated activation of the late complement cascade inside the GC (Yamakawa and Imai, 1992), in particular on FDCs (Halstensen *et al.,* 1988). It is intriguing to speculate that CD155 contributes to the protection of cells expressing complement receptors by trapping vitronectin on the cell surface.

The implication of cells of the immune system in PV pathogenesis was subject to intense discussions (Bodian, 1955; Sabin, 1956). The lymphatic tissue lining the gastrointestinal tract has been suggested to represent the primary site of an oral infection with PV (Bodian, 1955). Although virus propagation inside Peyers patches and tonsils is considered necessary to establish the viremic stage of an infection, the cells supporting virus growth have not been identified thus far. The results presented here restrict the repertoire of candidate cells to FDCs and follicular B cells comprising GCs, thus substantiating the importance of the immune system in initiating a PV infection. GCs do not belong to the structures regularly found inside secondary lymphoid organs. Indeed, we frequently obtained tissue from tonsillectomies lacking GCs and consequently never observed any staining specific for CD155 in such tonsils. This finding suggests that an already *ongoing secondary* immune response promotes the onset of a successful PV infection of the lymphoid organs surveying the alimentary tract. In contrast there is no indication that innate and primary immune responses are affected by the viral infection. Remarkably, only the small subset of GC B cells was found to express CD155. B cells residing in B cell zones and primary follicles mediating primary immunity lack CD155, thereby not supporting viral replication. Therefore, this pool of B lymphocytes, including those circulating in the blood stream (Freistadt *et al.,* 1993), do not contribute to virus dissemination as observed in the case of Type B coxsackieviruses (Mena *et al.,* 1999).

The study of the CD155 promoter and the transcription factors regulating its activity led us to suspect expression of CD155 during embryonic development (Solecki *et al.,* 1997, 1999, 2000). Recent analyses of the *CD155* promoter in the context of *CD155/β-gal* transgenic mice have revealed transgene expression in notochord, floor plate, and those regions of the ventral spinal cord fostering the developing motor neurons (Gromeier *et al.,* 2000). These locations are also sites of elevated vitronectin expression and it has been suggested that vitronectin can promote the differentiation of precursor cells into motor neurons (Martínez-Moralez *et al.,* 1997). A CD155/vitronectin interaction may thus serve different functions depending on the distinct site of their colocalization.

Unlike nectins, CD155 is not a homophilic cell adhesion molecule (Aoki *et al.,* 1997, our own unpublished data). We propose that CD155 mediates cell-to-matrix contacts via a strong and rapid interaction with vitronectin (see Table 1). Remarkably, the most N-terminally located V domains of the corresponding receptors are the mediators of their respective functions as discussed here (see Fig. 1 for sequence conservation; our own results and Miyahara *et al.,* 2000). The functional diversification of CD155 family members is also reflected by the nature of their cytoplasmic domains, which exert no homology at all. Whereas nectins have the propensity to interact with afadin, the cytoplasmic domain of CD155α specifically binds to tctex (S. Müller, X. Cao, and E. Wimmer, in preparation), a protein component of the cytoplasmic dynein complex (Hirokawa, 1998; King *et al.,* 1996). Hence CD155 may utilize a different pathway(s) to transmit extracellular binding events to the cell's interior.

MATERIALS AND METHODS

Cell culture

CHO, HEp 2, and HEp 2-Cl32 cells were grown in DMEM/10% FBS. HEp 2 cells express CD155 (Calvez *et al.,* 1995), whereas HEp 2-Cl32 cells, a subclone of HEp 2 kindly provided by F. Colbère-Garapin (Institut Pasteur, Paris), are devoid of CD155 expression. Sf9 cells were cultivated in Excell 401 (Biozol)/10% FBS.

Cloning and expression of soluble variants of CD155 and PRR2 (sCD155d1d3 and smPRR2d1d3)

The primers used for PCR generating sCD155d1d3 and smPRR2d1d3 (Fig. 1) were 5′-PVR (5′-CGGAAGATCTGGCATGGCCCGAGC-3′), 3′-PVRHIS (5′-GCTCTAGATTCAATGGTGATGGTGATGGTGCTCACTGGGAGG-3′), 5′-MPH (5′-CCAGATCTGGCATGGCCCGGGCCGCAGTCC-3′), and 3′-MPHHIS (5′-GCGAATTCAATGGTGATGGTGATGGTGATGGTGATGGTGATCTCGGGAGGCCTGGGGG-3′). PCR conditions were 3 min at 95°C; 5 cycles of 95°C for 45 s, 50°C for 1 min, 72°C for 2 min; 30 cycles of 95°C for 45 s, 55°C for 45 s, 72°C for 2 min, and 72°C for 5 min in a 50-μl reaction volume using CombiPol Polymerase (InViTek). The sCD155d1d3 (smPRR2d1d3) fragment was cut with *Bgl*II/*Xba*I (*Eco*RI/*Bgl*II) and cloned into the vector pVL1392 (PharMingen). Positive clones were sequenced and transfected along with linearized baculovirus DNA into Sf9 insect cells using the BaculoGold Transfection kit (PharMingen). Recombinant virus was recovered from the supernatant after 6 days at 27°C and passaged several times in order to produce a high-titer stock solution. For protein purification, insect cells were harvested 3 days postinfection and the cells lysed in 10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100. The cleared lysate was incubated with NiNTA-agarose (Qiagen) at 4°C several hours, the beads were washed five

times (50 mM NaP_i, pH 8, 300 mM NaCl), and the bound material was eluted with 50 mM NaP_i, pH 6, 300 mM NaCl, 500 mM imidazole. Buffer was exchanged using PD-10 columns (Pharmacia). Each preparation was checked for purity by SDS–PAGE and subsequent silver staining (Pharmacia).

Cloning and expression of a soluble, underglycosylated deletion variant of CD155 (Fig. 1; sCD155d1d2aglc)

Primers used for PCR were 5′-CD155, 5′-AACTGCAGATGGCCCGAGCCATGGCC-3′, and 3′-CD155, 5′-AACTGCAGTTAATGGTGATGGTGATGGTGCACAGTCAGCAGCTGAGGC-3′. A CD155 receptor construct used in earlier studies (Bernhardt *et al.,* 1994b), lacking four of five glycosylation sites in domains 1 and 2, served as template. The PCR product was cut with *Pst*I and cloned into pED4 (kindly provided by Genetics Institute). Plasmid DNA was transfected into DHFR-deficient CHO cells; a positive cell clone was selected and grown using methotrexate at a final concentration of 200 nM. Protein was purified from supernatant as described above.

Plaque reduction assay

This assay was done as described previously (Cao and Wimmer, 1996). In brief, 10,000 pfu of PV1 (Mahoney) was incubated with or without 10 μg purified sCD155d1d3 for 3 h at 4°C followed by 1 h at −37°C. Tenfold dilutions were then plaqued on HeLa cells according to standard procedures.

Cell adhesion assay

According to the instructions of the manufacturer, 96-well plates were coated with 500 ng human vitronectin (Promega), BSA (Sigma), or human collagen type IV (Gibco) per well for 1 h at room temperature. Wells were blocked with 2 mg/ml BSA in PBSd (8 g/l NaCl, 0.2 g/l KCl, 1.15 g/l Na_2HPO_4, 0.2 g/l KH_2PO_4) for another hour at room temperature. HEp 2 or HEp 2-Cl32 cells were detached using PBSd, 10 mM glucose, 2 mM EDTA and washed with serum-free DMEM. Cells (5×10^4 in a total volume of 100 μl) were added to each well and allowed to sit for 1 h at 37°C. For inhibition experiments, cells were preincubated for 5 min in the presence of the indicated proteins (10 μg 6C23/ml, 50 μg D171/ml, 50 μg P44/ml, 50 μg control mAb/ml, 10 μg sCD155d1d3/ml, 10 μg sCD155d1d2aglc/ml) before the cells were added to the wells. Wells were then washed three times with DMEM and bound cells quantified using the CellTiter 96 cell proliferation assay (Promega).

Dot blot assays

One microgram of protein each of BSA, human collagen type IV, and human vitronectin (see also previous paragraph) was spotted onto nitrocellulose membranes (Amersham). All steps were performed at room temperature. The membrane was blocked with 2 mg/ml BSA, washed two times with TBST (Tris–HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20), incubated for 10 min with 1 μg sCD15d1d3 in TBST, and washed three times with TBST before the bound sCD155d1d3 was detected using the PentaHis antibody (Qiagen) followed by an anti-mouse antibody coupled to alkaline phosphatase (Sigma). After three washes with TBST the blots were developed with the NBT/BCIP system (Promega) and quantified densitometrically.

BIAcore measurements

Surface plasmon resonance measurements were carried out with a BIAcore instrument (Biacore). Proteins [vitronectin from rat (Sigma) or human (Promega), BSA, D171, smPRR2d1d3, and human collagen type IV (Gibco)] were immobilized in 10 mM Na acetate, pH 5, on a CM5 sensor chip using standard EDC (1-ethyl-3-(dimethylaminopropyl)carbodiimide-HCl) and NHS (*N*-hydroxysuccinimide) coupling chemistry and subsequent saturation of free binding groups with ethanolamine. Running buffer was HBS (Biacore). For analysis typically a sample volume of 100 μl was injected at a flow rate of 10 μl/min. A coating density of approximately 2000 relative units was loaded in each case. Calculations were done using the Biaevaluation 2.0 software assuming a simple first-order reaction.

Immunohistochemistry

For immunohistochemical analyses, human tonsils from routine tonsillectomies (kindly provided by Dr. Fink, Berlin-Buch) were embedded in Tissue Tek (Miles) and snap frozen in liquid nitrogen. Cryostat sections of a thickness of 10 μm were dried overnight on gelatin-covered slides and fixed with acetone. Slides were rehydrated and blocked for 1 h in staining buffer (100 mM Tris–HCl, pH 8, 150 mM NaCl, 0.1% Tween 20) supplemented with 5% rat serum. The sections were incubated in staining buffer containing the corresponding antibody for 1 h in each step. CD155 was detected using mAb D171 and biotinylated anti-mouse IgG antibody as secondary antibody. For single stainings, the signal was enhanced using the tyramide signal amplification system Direct Green (NEN DuPont) and for double stainings with the tyramide signal amplification system Direct Red (NEN DuPont). The anti-myc mAb 9E10 was used as a control. For the detection of vitronectin, the mAb clone Vn5-3 (TaKaRa) was used and bound antibody was detected with an anti-mouse IgG mAb labeled with either FITC or TRITC (Jackson). A pentaHIS mAb (Qiagen) served as a control antibody. In the case of the double staining of CD155 and vitronectin, the latter was performed first followed by an incubation with biotinylated

mAb D171 in order to detect CD155 (including signal amplification as outlined above). FDCs were stained with directly labeled mAb HJ2 (Sigma, and a kind gift from M. H. Nahm), whereas GC B cells were detected with PNA–FITC (Vector) in a detergent-free buffer system (10 mM HEPES, pH 7.5, 150 mM NaCl, 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$) (Butch and Nahm, 1992). Staining with PNA was always done at the end of a staining sequence. For the HJ2 antibody, an irrelevant IgM–FITC conjugate (Cymbus) was used as a control. The anti-CD3 mAb (UCHT-1; Dianova) conjugated with biotin was used for staining T cells. Bound mAb was visualized with streptavidin–FITC. Detection of CD51 (N-19; Santa Cruz) was performed as described for CD155 using the tyramide signal amplification system. The slides were mounted in Moviol (Calbiochem) and analyzed by confocal laser microscopy (Leica, TCS 4D-I).

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