Clearly, the absolute number of substitutions alone does not signify attenuation, because the fully attenuated Zagreb strain has fewer substitutions, compared with Edmonston wild-type than Rubeovax. Figure 3 demonstrates that the 2 most divergent strains are Shanghai-191 and CAM-70 (89 nucleotide differences from each other or 0.6% of the genome), which were derived from unique wild-type progenitors. These 2 strains also have the largest number of nucleotide differences from Edmonston wild-type. This degree of divergence is likely due to their generation from non-Edmonston progenitors; however, this interpretation cannot be tested until the sequences of their progenitors become available. The most closely related strains are Rubeovax and Moraten or Rubeovax and Schwarz (16 nucleotide differences, respectively). A surprising finding was that Moraten and Schwarz have identical nucleotide sequences, despite their divergent passage histories [95, 96]. Because both viruses have been passaged in CEF at reduced temperatures (Figure 2), it is possible that similar cell culture conditions may have resulted in similar nucleotide substitutions. Despite the diverse geographic origins of the progenitors and the variations in cell culture systems, incubation temperatures, and passage numbers, the genomes of vaccines demonstrate remarkable sequence similarity. Phylogenetic analysis (Figure 3) indicated that Changchun-47 could have been derived from Leningrad-4, which is consistent with the strain's reported passage history [115]. The increased number of nucleotide substitutions in CAM-70 is distributed throughout the coding sequences of the genome rather than the noncoding sequences (Figure 4). All genes—but especially the P/C/V gene— are affected, whereas the variability of the M gene is similar to that of the other vaccines. We performed an analysis of synonymous versus nonsynonymous substitution rates [121], but the results were meaningless for many ORFs that had either no synonymous or no nonsynonymous substitutions (eg, in the C and H ORFs) (Figure 4).

Variability in Individual Proteins

Table 1 lists the nucleotide and amino acid substitutions that are specifically discussed in the text. For a complete list of substitutions in the genomes of measles vaccines, see Supplemental Table 1. With the exception of Shanghai-191, the N genes of the vaccine strains show much higher conservation than the P/V/C, M, and H genes. Comparable to the situation in wild-type isolates [122], N_{CORE} is much more conserved among the vaccines (only 4 cumulative nucleotide changes in the region coding for the amino terminal 400 amino acids for all the vaccines) than N_{TAIL} (12 nucleotide changes in the region coding for the carboxyl terminal 125 amino acids) (Supplemental Table 1). Although the number of substitutions in Shanghai-191 is generally similar to that of other strains (except CAM-70), the N gene of Shanghai-191 is considerably more divergent than the N genes of all other viruses (Figure 4). The reason for the variability of the Shanghai-191N gene is that it accumulated 8 T→C changes, all of which are unique to Shanghai-191 (Supplemental Table 1). All of the T→C changes are located in the hypervariable N_{TAIL} region, and 2 of them predict substitution with Pro residues that may affect protein conformation. The T→C changes may be an example of hypermutation in a vaccine strain. Hypermutation has been described for sequences of M genes derived from patients with SSPE [123]. The effect has been attributed to the action of the

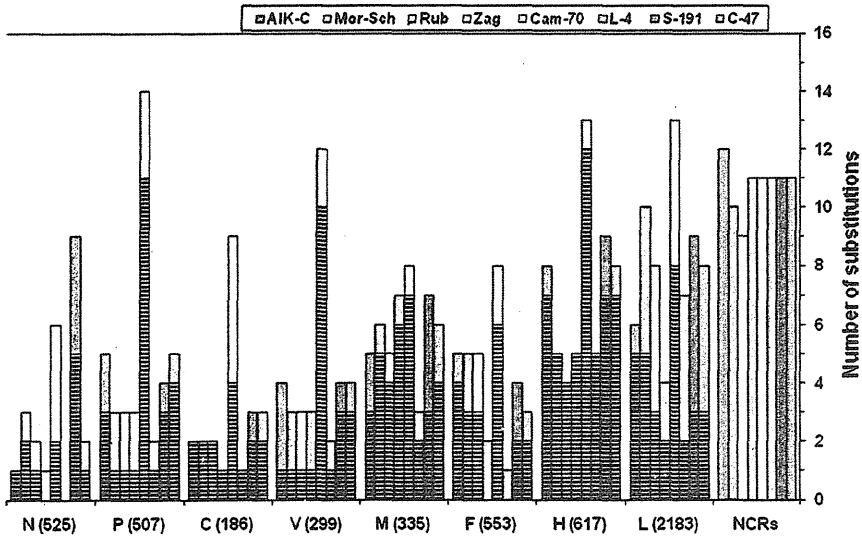

Figure 4. Number of nucleotide and predicted amino acid changes in the coding and noncoding regions of measles vaccine strains, compared with Edmonston wild-type strain. The number of amino acids is listed in brackets behind each protein. Horizontally striped bars represent coding changes, and open bars represent noncoding or silent changes. NCR, noncoding region.

Table 1. Nucleotide and Amino Acid Substitutions Discussed in the Text

Gene	Nucleotide	Edmonston wild-type	Vaccine	Amino acid position	Edmonston wild-type	AIK-C	Mor-Sch	Rub	Zag	CAM-70	L-4	S-191	C-47
	26	A	T	—	—	●	●	●	●	●	●	●	●
	42	A	C	—	—	●	●	●	●	●	●	●	●
N	722	A	G	—	—			●					
P	1806	G	A	—	—		●	●		●			
	2046	C	T	—	—	●	●	●	●	●	●	●	●
	2063	G	T	86	S							I	
	2101	A	G	99	N					D			
	2134	T	C	110	Y								H
	2135	A	G	110	Y					C			
	2167	A	G	121	K					E			
	2480	A	G	225	E	G	G	G	G	G	G	G	G
	3122	T	C	439	L	P							
V	**2046**	C	T	—	—	●	●	●	●	●	●	●	●
	2063	G	T	86	S							I	
	2101	A	G	99	N					D			
	2134	T	C	110	Y								H
	2135	A	G	110	Y					C			
	2167	A	G	121	K					E			
	2480	A	G	225	E	G	G	G	G	G	G	G	G
C	**2046**	C	T	73	A	V	V	V	V	V	V	V	V
M	**3619**	G	A	61	G	D	D	D	D	D	D	D	D
	3627	C	T	64	P		S	S		S			
	3702	G	A	89	E	K	K	K	K	K	K	K	K
	4232	G	A	—	—	●	●	●	●	●	●	●	●
	4608	T	C	—	—			●					●
F	**4978**	T	C	—	—	●	●	●	●	●	●	●	●
	5073[a]	C	G	—	—	●	●	●	●	●	●	●	●
	5308	T	C	—	—			●					
	5349	T	C	—	—	●	●	●	●	●	●	●	●
	5514	G	A	—	—	●	●	●	●	●	●	●	●
	6542	C	A/T[b]	362	S	Y (A)	Y (A)	Y (A)		F (T)			
	6743	C	T	429	T			I					
	7243	T	C	—	—		●						
H	**7407**	C	T	46	S	F	F	F	F	F	F	F	F
	7901	A	G	211	S	G	G	G	G	G	G	G	G
	8711	A	T	481	N	Y	Y	Y	Y	Y	Y	Y	Y
	8721	C	A	484	T	N			N		N	N	N
	8906	G	A	546	G	S	S	S		S	S	S	S
L	9936	A	G	235	I					V			
	12,600	A	C	—	—			●					
	14,383	A	C	1717	D	A	A	A	A	A	A	A	A
	14,579	A	G	—	—	●	●	●	●	●	●	●	●

NOTE. For abbreviations of virus names, see Figure 3. Dashes denote noncoding region, empty fields denote no change, compared with Edmonston wild-type, and dots denote substitution in noncoding region. Nucleotides highlighted in bold indicate a substitution in at least 8 vaccine strains.

[a] Correction to GenBank entry: Nucleotide 5073 in the GenBank entry for Edmonston wild-type (AF266288) is a G; the nucleotide was determined to be a C, as reported elsewhere [95] (B. Bankamp, unpublished data).

[b] Nucleotide positions with a mix of substitutions list the nucleotide in brackets behind the amino acid. For a list of all nucleotide and amino acid substitutions, please see Supplemental Table 1.

cellular double-stranded RNA-dependent adenosine deaminase [124], and the resulting defects in M protein function or expression contribute to the cell-associated growth pattern of SSPE viruses [125]. As the SSPE cases demonstrate, viruses with defective M proteins can replicate; however, the N protein is indispensable for replication. Although the N protein of Shanghai-191 is obviously functional, it is not known whether the large number of substitutions contributes to the strain's attenuation.

The Leucine residues that are important for the interaction of N with the M protein [21] are conserved in all strains. The N_{TAIL} regions of vaccine strains and Edmonston wild-type possess Asn522, whereas currently circulating wild-type strains mostly possess Asp522 [29]. Asn522 is important for interaction with heat shock protein 72 (hsp72) [29]. This difference appears to reflect differences between genotypes rather than between vaccine and wild-type strains, because other genotype A, wild-type isolates share an Asn at position 522 (GenBank accession numbers AF045218 and AY647965). Although a recombinant MeV possessing Asp522 demonstrated reduced fitness in cell culture and cotton rats [29], the fact that current patient isolates share the Asp at this position indicates that these model systems may not reproduce all fitness pressures that wild-type viruses experience in the natural host. In addition to hsp72 and the M protein, N_{TAIL} interacts with other binding partners, including several cellular proteins; however, no specific amino acids that are important for the interactions have been identified.

Nucleotide substitutions are often observed in the P gene after passages of MeV in cultured cells, and the P genes of wild-type viruses demonstrate a higher level of variability than the corresponding N genes [126–131]. Remarkably, 35 of 42 cumulative changes in the P gene are located in the amino-terminal domain shared between the P and V proteins (Supplemental Table 1). The carboxyl termini of both P and V are well conserved. These observations agree with data from wild-type MeVs in that the shared amino terminal domain of V and P was found to be more variable than the carboxyl termini in wild-type MeV isolates [132]. The unique carboxyl termini of P and V are required for replication and for control of the innate immune response, respectively; the higher level of conservation indicates that these regions are less tolerant of amino acid substitutions. Two amino acid substitutions, Tyr110His and Cys272Arg, which are found in the common amino-terminal region of the P and V proteins and the unique carboxyl-terminal region of the V protein, respectively, disrupt the abilities of the V and P proteins to counteract the IFN signaling pathways [34, 36, 43, 133]. Adaptation of a wild-type virus to chick embryo fibroblasts introduced the Tyr110His substitution, indicating that this substitution may be important for improved growth in avian cells [127]. The P/V proteins of the Changchun-47 vaccine possess Tyr110His, and the P/V proteins of the CAM-70 vaccine possess Tyr110Cys. The latter substitution reduced the ability of the CAM-70 V protein to counteract IFN signaling [43]. That the same position was changed in 2 independently derived vaccine strains through different nucleotide substitutions may indicate the importance of impeding IFN resistance in vaccine attenuation. The P protein (and presumably also the V protein) is phosphorylated at several residues in its amino-terminal domain, including amino acids 86, 151, and 180 [134]. The P and V proteins of Shanghai-191 possess a Ser86Ile substitution, which would abolish phosphorylation of this residue. The effect that this potential change in phosphorylation may have on the functions of P and V is currently unknown. One substitution that affects all vaccine strains is located at nucleotide 2480 in the P gene. This not only introduces a nonconservative Glu225Gly substitution at the end of the shared P/V domain—it also is located only 11 nucleotides upstream of the editing site. However, there was no significant difference in the frequency of P and V ORFs in transcripts from viruses with either nucleotide at position 2480 [132].

The P protein of CAM-70 varies from Edmonston wild-type at 11 amino acid positions and the V protein in 10 positions, while other vaccines vary at no more than 4 positions for the P protein and 3 for the V protein (Figure 4 and Supplemental Table 1). Many of these substitutions are nonconservative changes, including charge-switches at amino acids 99 and 121. Although the effect of the substitution at amino acid 110 in the P/V protein of CAM-70 on the inhibition of IFN signaling has been demonstrated [43], the contribution of the many other substitutions to attenuation remains to be investigated. The P protein of AIK-C renders the virus temperature-sensitive [135]; a Leu439Pro substitution was found to be one contributing factor [136]. None of the other vaccines share this substitution.

There are a cumulative total of 25 nucleotide substitutions and 18 amino acid changes in the small C ORF (186 amino acids), with a high proportion of coding changes (72%) (Supplemental Table 1). Such a high level of variability may indicate the importance of the C protein as a virulence factor. The C ORF of CAM-70 has 9 nucleotide changes, compared with only 0-3 in the other vaccines; however, the number of silent changes is high (Figure 4). As a result, the number of amino acid substitutions in CAM-70 C is 4, whereas the other vaccines have 0-2 substitutions. The high number of silent changes in the CAM-70 C ORF is a result of coding changes in P and V proteins. The C protein interferes with the induction of IFN and apoptosis through down-regulation of transcription [35, 42]. Using a mini-replicon assay system, Bankamp et al [45] reported that the inhibitory activity of the C protein toward viral RNA synthesis differed among 6 MeV strains and that substitutions in the region between amino acids 46 and 167 of the C protein affected its ability to down regulate viral transcription. All but 1 of the substitutions in the C proteins of the vaccine strains are located in this region (Supplemental Table 1), so it is possible that some of them may affect the ability of the C protein to control the induction of IFN. After adaptation of a wild-type virus to CEF, the C protein contained 1 amino acid change that affected its ability to down-regulate transcription [126], providing additional evidence of the importance of the C protein in cell-culture adaptation. Disturbing the delicate balance between the rate of replication and inhibition of host defenses may lead to attenuation. Because the C proteins are important virulence factors [90, 137–140], the differences among the C proteins may also contribute to the attenuated phenotypes of certain measles

vaccines. However, the C proteins of some vaccine strains remain fully functional. Nakatsu et al [47] compared the activities of C proteins from wild-type and vaccine strains using reverse-genetics techniques and found that a C protein identical to that of Zagreb equally regulated viral RNA synthesis, compared with the wild-type C protein, and was capable of circumventing the host IFN induction. Clearly, impaired function of the C protein is not a prerequisite to attenuation. The Ala73Val substitution is shared by all vaccine strains, including those derived from non-Edmonston progenitors. However, wild-type viruses from genotypes B3 and D3 also have Val at this position [132], making it less likely that the substitution has consequences for attenuation.

The role of the P/V/C gene in attenuation is well documented with different animal models. Recombinant viruses that do not express a V or a C protein are attenuated in rhesus macaques [139, 141]. A CEF-adapted virus with mutations affecting the P, V and C proteins as well as a substitution in M did not cause rash in macaques [127]. Takeda et al [142] showed that a recombinant MeV possessing the P gene of the Edmonston-tag strain exhibited attenuated gene expression levels in both cultured cells and mice expressing the hSLAM receptor. In cotton rats, recombinant viruses carrying the temperature-sensitive P gene of AIK-C were unable to replicate after intranasal inoculation [143].

The M gene has 50 cumulative changes in only 1008 nucleotides, of which 39 (78%) are coding changes, indicating both a higher overall substitution rate and a higher proportion of coding changes than the N, F, or L genes (Supplemental Table 1). Tahara et al [144] showed that the M protein derived from the wild-type IC-B strain associated with the cytoplasmic tail of the F protein, but it associated poorly with the cytoplasmic tail of the H protein. They further indicated that Pro64Ser and Glu89Lys substitutions allow a strong interaction of the M protein with the cytoplasmic tail of the H protein [144]. All measles vaccines with published sequence data possess the Glu89Lys substitutions in the M protein. The independently derived CAM-70 vaccine shares the Pro64Ser substitution with the Edmonston-derived Rubeovax, Moraten, and Schwarz, indicating the importance of this change. Vero cells do not efficiently express entry receptors for wild-type MeV. These substitutions allow wild-type MeV to grow well in Vero cells, presumably by enhancing MeV assembly and subsequent infectious particle production [144]. On the other hand, Pro64Ser and Glu89Lys inhibit receptor-dependent cell-cell fusion, thereby reducing virus growth in hSLAM-positive cells [145]. These data indicate that the Pro64Ser and Glu89Lys substitutions modulate the mode of MeV growth for adaptation to grow in different cell types. However, the contribution of the substitution at amino acid 89 to attenuation is unclear, because a wild-type MeV with the Glu89Lys substitution grew to higher titers in cotton rats than did the wild-type. [146]. In addition to the Glu89Lys substitution, all vaccine strains share a Gly61Asp substitution that is close to the important position of amino acid 64; however, no functional consequence is known for this substitution.

The F glycoprotein F is more conserved than the M or H genes, and the proportion of coding changes is lower (61%) (Supplemental Table 1). There are few differences between the F proteins of the prototype Edmonston strain and current wild-type strains [130, 131]. Importantly, the amino acid sequence of the F protein of the attenuated Zagreb strain is identical to the Edmonston wild-type F protein, indicating that large numbers of passages in various cell culture systems did not lead to adaptive changes. Furthermore, the Zagreb F protein is identical to the more recent wild-type IC-B isolate, a well-characterized virulent strain [131]. This demonstrates the high level of conservation in the F protein. One reported amino acid substitution that affects F protein function is Met94Val. F proteins possessing the Met94Val substitution exhibit enhanced cell-cell fusion activity [147–149]; however, Edmonston wild-type and all vaccine strains share the Met at this position. Edmonston-derived vaccines AIK-C, Schwarz, Moraten, and Rubeovax share a Ser362-Tyr substitution, whereas the independently derived CAM-70 has a Ser362Phe substitution. That the same position was changed in independently derived vaccine strains through different nucleotide substitutions may indicate the importance of this residue. Not surprisingly, important functional domains and motifs, such as the cytoplasmic tail, cleavage site, and fusion peptide, are completely conserved.

Similar to the M gene, the H gene has a very high ratio of coding changes (92%), indicating that these may be driven by selection. A large body of research is available for the study of receptor binding by measles H proteins [65]. Not surprisingly, all amino acid positions that are involved in binding to hSLAM and EpiR are conserved in Edmonston wild-type and all vaccine strains [72, 74, 150, 151]. Several amino acid positions that are involved in the ability of MeV strains to use CD46 have been reported [152–158]. Among these, the Asn481Tyr substitution is critical for use of CD46 as a receptor [152–154, 158–162]. The H proteins of all vaccine strains possess the Asn481Tyr substitution. However, this substitution alone does not confer efficient use of the CD46 receptor [163]. Tahara et al [164] showed that a Glu492Gly or Thr484Asn substitution, when present together with Asn481Tyr, enhances the ability of the wild-type H protein to use CD46. AIK-C, Zagreb, Leningrad-4, Shanghai-191, and Changchun-47 possess Thr484Asn, presumably enhancing their ability to bind CD46. Independently of Asn481Tyr, a Ser546Gly substitution can confer on MeV the ability to use CD46 as a receptor, and some Vero cell-grown MeV strains have the Ser546Gly substitution in their H proteins, instead of Asn481Tyr [129, 143, 156, 159, 160, 165–170]. The H protein of the Zagreb vaccine strain possesses Ser546Gly; however, so does the Edmonston wild-type. A recent study of the crystal structure of an MeV H protein complexed with CD46 also indicated critical roles of Asn481Tyr and Ser546Gly [151].

Interestingly, the Asn481Tyr substitution that is critical for using CD46 also promotes MeV infection of polarized epithelial cells via a CD46-independent pathway [74]. Therefore, all vaccine strains which possess Asn481Tyr in their H proteins may be able to use EpiR more efficiently than can wild-type MeV strains. A Val451 has been reported to improve CD46 binding, and all vaccine strains and the Edmonston wild-type strain share this amino acid. The fact that the Edmonston wild-type strain possesses amino acids corresponding to the cell culture–adapted phenotype may be evidence of some degree of cell culture adaptation. A Gly211 was reported to be involved in CD46 receptor down-regulation [152]. All vaccines share Gly211, in contrast to the Edmonston wild-type strain, which has a non–down-regulating Ser211. Receptor down regulation may be associated with protection of the infected cell from complement lysis [171]. In total, there are 3 amino acid substitutions in the H protein that are shared by all vaccine strains (amino acids 46, 211, and 481) and 1 additional substitution shared by all but the Zagreb strain (amino acid 546). Three of these 4 positions may play a role in CD46 binding or down-regulation, demonstrating the importance of receptor adaptation for growth of vaccines in hSLAM-negative cell lines. The functional consequence of the nonconservative Ser46Phe, which is located in the hydrophobic transmembrane domain [172], is unknown. The H proteins of some currently circulating wild-type strains have an additional glycosylation site, owing to an Asp416Asn substitution [62, 169, 173]. Although the glycosylation site at this position is located on the opposite side to the CD46-binding region [174], it has also been shown to modulate the CD46-binding activity of the H protein [164]. However, none of the vaccine strains possess this substitution. The cytoplasmic tails of the H proteins (amino acids 1-34) are completely conserved.

Considering its large size, the L gene is considerably more conserved than the other genes, with only 75 cumulative changes in 6549 nucleotides, of which only 36 (48%) are coding changes (Supplemental Table 1). This observation is consistent with previous analyses [96, 175]. The conservation of the L gene demonstrates that substitutions in the polymerase are deleterious for the virus and therefore not tolerated. On the basis of sequence conservation, 6 domains have been characterized for the L proteins of paramyxoviruses [83]. Eleven of the 16 positions that vary among the vaccine strains are located between the conserved domains, and 10 of these affect residues that are not conserved among the polymerases of nonsegmented negative strand viruses [81]. Of the 5 substitutions that affect residues within conserved domains, 3 affect residues that are conserved among polymerases. Ile235Val lies in a highly conserved hydrophobic stretch in domain I; however, it is a substitution that conserves the hydrophobic nature of the residue. Aside from this substitution, all highly conserved stretches and motifs [81] are completely conserved. One substitution (Asp1717Ala) is shared by all vaccine strains; however, several other wild-type strains in genotype A also have an Ala at this position [175]. Therefore, this is more likely to be a genotype-specific residue with Edmonston wild-type being the exception. Amino acids 1-408 of L are involved in L oligomerization, and hydrophobic amino acids between positions 25 and 339 are involved in binding to the P protein [82]. Four residues in this region are variable between vaccine L proteins, and 3 of these substitutions affect CAM-70 (Supplemental Table 1). Because interactions between the components of the polymerase complex are essential for virus replication, clearly these substitutions cannot inhibit L-L or L-P interactions. Whether they have subtle effects on these interactions is currently unknown. On the basis of investigations with recombinant MeV, the L gene of an attenuated laboratory strain contributed to the reduced gene expression and virus propagation in cell culture and in mice expressing hSLAM [142]. However, reduced gene expression was not observed when vaccine L proteins were used in a mini-replicon assay [142]. In addition, the L genes of measles vaccine strains showed higher activities than did the L genes of some wild-type MeV strains when analyzed by mini-replicon assays [176]. Therefore, the contribution of the L protein to attenuation remains unclear.

Variability in Noncoding Regions

With 1 exception (nucleotide 7243, see below) all known cis-acting elements involved in transcription and replication (ie, promoter elements, intergenic regions, and gene-start and -end sequences) were conserved, as was the entire 37 nucleotide trailer sequence [2, 177]. The high level of conservation of trailer sequences of vaccine strains and wild-type viruses of different genotypes had already been reported [178]. Two nucleotide changes (26 and 42) in the leader sequence distinguish Edmonston wild-type from all vaccine strains. Although all vaccines share the A→T change at nucleotide 26, the substitution at nucleotide 42 is A→C for all vaccines except Zagreb, which has an A→T substitution. These positions affected transcription of a reporter gene in a mini-genome assay [178], and it is conceivable that they may affect replication of vaccine viruses. However, Liu et al [178] also reported that wild-type MeV isolates of genotype A had the same substitutions as the vaccine strains, so the relevance of these positions for attenuation is still unclear. In addition, no effect was observed when these substitutions were introduced into the infectious virus genome [142]. A substitution in Moraten, Schwarz, Rubeovax, and CAM-70 at nucleotide 1806 immediately precedes the AUG start codon of the P protein, possibly affecting translation efficiency [179]. It is intriguing that this substitution was selected for in Edmonston-derived and independently derived vaccines. However, the context of all MeV P start codons deviates from the optimal Kozak sequence [179], making it difficult to assess the effect of the substitution at nucleotide 1806. The suboptimal Kozak context of the P ORF possibly serves to enhance initiation at the downstream start codon for the overlapping C ORF, and it

is conceivable that substitutions that alter P protein translation could affect C protein translation.

Forty-eight nucleotide changes (affecting 19 positions) were located in the long untranslated region spanning the end of the M and the beginning of the F gene (Supplemental Table 1). The fact that MeV, like other morbilliviruses [56, 87], maintains this noncoding region of ~1 kB indicates that this region may play an important role, but its function is still unclear. It is possible that substitutions to this region may affect mRNA structure or stability. Changes to the long untranslated 3′ region of the M gene can increase M protein expression [89]. Deletion of the F nontranslated region reduced viral replication in a SCID mouse model [90] and nucleotide changes in this region decreased F protein expression [89]. Although the implications of single nucleotide changes in the F 5′ noncoding region are unclear, it is intriguing that 3 positions (4978, 5073, and 5349) in this region are changed in all vaccine strains, including those derived from non-Edmonston progenitors.

The only substitution that affects a conserved motif is a substitution in the gene-end signal of the F gene of Moraten and Schwarz (nucleotide 7243). However, although this nucleotide is part of the gene-end motif, this particular position is not conserved among the gene-end signals of MeV [95], making it less likely that the substitution changes transcription.

Substitutions That Affect Most Vaccine Strains

Nucleotide substitutions, whether silent or not, that affect most vaccine strains are particularly interesting candidates for attenuation markers, especially if they are shared between Edmonston-derived and independently derived strains. There are 17 such changes among the vaccine strains analyzed to date (Table 1): 16 that affect all vaccine strains, and 1 (amino acid 546 in the H protein) that affects all vaccines except Zagreb. Three of these are silent nucleotide changes in coding sequences (nucleotides 4232, 5514, and 14579). Five affect noncoding regions (nucleotides 26, 42, 4978, 5073, and 5349), whereas the remaining 9 predict coding changes in P, V, C, M, H, and L proteins. Several of these substitutions have demonstrated functional consequences, at least in in vitro systems, including nucleotides 26 and 42 as well as amino acids 89 in the M protein and amino acids 211, 481, and 546 in the H protein. However, functional consequences of these substitutions could not always be confirmed in animal models [142, 146]. Because attenuation is generally assumed to be the result of an accumulation of mutations, it is possible that introduction of 1 or a few changes at a time will not be sufficient to produce measurable differences in pathogenicity in animal models.

GENETIC STABILITY OF VACCINE SEQUENCES IN CELL CULTURE AND VACCINEES

Borges et al [97] sequenced CAM-70 directly from the seed lot and after 10 passages in primary chicken embryo fibroblasts and found complete sequence identity. Baricevic et al [180] compared a master seed lot of Zagreb with a working seed and also found complete sequence identity. However, 6 nucleotide differences were found between their sequence and the sequence of Zagreb, as published by Parks et al [96, 97], that is used in this review [180]. Passages of the vaccines in the laboratories where they were used may account for those differences. Sequences derived from vaccine-associated measles cases usually only report the carboxyl-terminal region of the N protein which was chosen for genotype analysis. Analysis of 7 such sequences available on GenBank revealed complete nucleotide identity with Moraten/Schwarz (B. Bankamp, unpublished data).

SUBGENOMIC RNAs

Defective interfering particles (DIs) have been found in several vaccine strains, including Zagreb, AIK-C, and CAM-70 [181, 182]. DIs contain subgenomic RNAs which are generated during replication of the full-length genome [183]. Because of large deletions in the protein coding regions, they require coinfection of the cell with a full-length genome to provide viral proteins. DIs can interfere with the replication of the full-length genome, leading to reduced viral titers in cell culture [184]. Viral stocks that contain DIs induce higher amounts of IFN [185]. Whether the presence of DIs or the resulting increase in IFN induction play any role in attenuation is not known.

IMMUNOGENICITY, PROTECTION AGAINST DIFFERENT GENOTYPES, AND ADVERSE EVENTS

Measles vaccine is one of the most efficacious vaccines presently available [113]. Seroconversion rates have exceeded 98% in children aged ≥12 months for most vaccines studied [9, 103, 114–116, 186, 187]. These data lead to the conclusion that there are no significant differences between seroconversion rates of the different vaccine strains.

On the basis of the sequences of their N and H genes, MeVs can be assigned to 1 of 23 genotypes and 1 provisional genotype [11, 12]. All vaccine strains and their wild-type progenitors are assigned to genotype A. Experiments with monoclonal antibodies have defined antigenic differences between the H proteins of genotype A vaccines and the H proteins of wild-type viruses grouped in other genotypes [62, 188, 189]. However, there is only 1 serotype for measles, and serum samples from vaccinees neutralize viruses from a wide range of genotypes, albeit with different neutralization titers [188, 190] More importantly, despite the presence of different endemic genotypes, vaccination programs with standard measles vaccines have been successful in every country where they were performed adequately [191–193]. Suboptimal seroconversion after vaccination is likely the result of inadequate coverage; improper administration, transport, or storage of vaccine; or age of the vaccine recipients [194–196].

Measles vaccines have an outstanding safety record, with only very small numbers of adverse events reported. The most common adverse events are fever and rash beginning 8-12 days after vaccination. Most of these reactions resolve quickly without need of clinical intervention [113]. The insufficiently attenuated Leningrad-4 caused fever (temperature, >38.7°C) and rash in all 7 vaccine recipients [114], and Rubeovax caused fever (temperature, >38.2°C) in 46% of recipients and rash in 16% of cases [9]. The further-attenuated strains induced less severe and less frequent side effects, from 2% of vaccinees that presented with fever (temperature, >38°C) after vaccination with Zagreb to 21% of subjects with temperatures >38.2°C after vaccination with Schwarz [9, 103, 105, 114–116, 187, 197]. The occurrence of rash ranges from 3% in Moraten-vaccinees to 20% in AIK-C-vaccinees [9, 103, 116, 187, 198, 199]. Although there appear to be significant differences in the rates of rash and fever, most vaccines were tested in separate studies and under different conditions, and the cutoff values for fever varied from study to study. Vaccines can cause severe adverse events in immunocompromised individuals whose immune systems are incapable of clearing the virus [200–202]. Children who are human immunodeficiency virus seropositive but do not demonstrate immune impairment can be safely vaccinated with measles vaccine, and they derive some protection from wild-type virus [203, 204]. There is no evidence that measles vaccines are responsible for SSPE [205], because only wild-type viruses have been detected in these cases.

GENETIC BASIS FOR ATTENUATION

Attenuation is the result of adaptation of the virus to growth conditions in nonpermissive cell culture, in the case of measles especially avian cell lines. This scenario implies that many attenuating mutations affect interactions with host cell proteins. Presumably such mutations increase interaction with cellular proteins in avian cells yet reduce the ability of the protein to carry out its functions in its human host. Most experiments have relied on mammalian cells for the analysis of differences between wild-type and vaccine viruses, whereas few studies used avian cells [127, 206, 207]. Substitutions in the P, V, C, M, and H proteins have been most often associated with measurable differences between vaccine strains and wild-type strains. Specifically, receptor use and interactions with IFN induction/signaling have been shown to be important, as well as substitutions in the M protein that affect interactions with the nucleocapsid. The L proteins of vaccine viruses also appear to contribute to attenuation in ways that are not yet understood. However, no common functional change that affects all vaccines has been demonstrated. This may be because multiple, cumulative changes appear to be responsible for attenuation and because separate passage histories may have given each vaccine strain a different pathway to attenuation. Therefore, although we have made significant progress in analyzing functional differences between wild-type and vaccine viruses, the molecular basis of attenuation remains unknown. Almost every gene contributes in some way to a cell culture-adapted phenotype; this complicates the identification of the molecular basis for attenuation in humans. Recombination, which is an efficient means for rapid genetic change for many viruses, does not occur in paramyxoviruses. No recombinant viruses have been isolated from natural infections. One constraint that may explain the absence of recombination is the rule of 6 [85, 208]. Genome lengths in the subfamily *Paramyxovirinae* have to be evenly divisible by 6, and genomes with insertions or deletions that violate the rule of 6 would result in nonviable virus. The complex pathway to cell culture adaptation and, ultimately, attenuation together with the absence of recombination suggest that it would be extremely unlikely for measles vaccine strains to revert to a virulent phenotype.

Supplementary Data

Supplementary data are available at *The Journal of Infectious Diseases* online.

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Wild-type measles virus is intrinsically dual-tropic

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Measles is a highly contagious disease that causes temporary and severe immunosuppression in patients. Signaling lymphocyte activation molecule (SLAM) expressed on cells of the immune system functions as a receptor for measles virus (MV). In addition to SLAM, vaccine strains of MV also use a ubiquitously expressed complement regulatory protein, CD46, as a receptor, whereas wild-type (wt) MV strains do not use this receptor. However, recent studies have indicated that SLAM is not the sole receptor for wt MV strains. These strains have an intrinsic ability to enter both immune and epithelial cells using distinct receptor binding sites in their hemagglutinin (H) protein. Recently, a clear answer was obtained through the identification of an epithelial MV receptor, nectin4, expressed at adherens junctions, thereby greatly improving our knowledge of MV receptors. It is now clear that MV specifically targets two cell types, immune cells and epithelial cells, using SLAM and nectin4, respectively. MV loses the ability to use either SLAM or nectin4 when it possesses specific mutations in the H protein. However, nectin4-blind MV still infects SLAM-positive immune cells efficiently (SLAM-tropic), and conversely, SLAM-blind MV infects nectin4-positive epithelial cells efficiently (nectin4-tropic). In this regard, MV is intrinsically dual-tropic to immune cells and epithelial cells. Although many aspects and molecular mechanisms underlying immunosuppressive effects and a highly contagious nature of MV still remain to be elucidated, analyses of physiological functions of these two receptors would provide deep insights into MV pathogenesis.

Keywords: measles virus, dual-tropic, SLAM, nectin4, receptor

MEASLES VIRUS

Measles is a highly contagious acute viral disease characterized by high fever, malaise, coryza, conjunctivitis, cough, and a maculopapular rash (Griffin, 2007). Patients with measles develop a severe and temporary immunosuppression, which is often accompanied by secondary bacterial infections (Griffin, 2007). Despite the availability of highly effective vaccines, measles-related deaths were estimated to be 164,000 worldwide in 2008 (WHO, 2009). The causative agent is measles virus (MV), which belongs to the genus *Morbillivirus* in the family *Paramyxoviridae*. The virus particle is enveloped and contains a non-segmented negative-strand RNA genome encoding six tandem linked genes, N, P/V/C, M, F, H, and L. The genome is encapsidated by the nucleocapsid (N) protein and associated with viral RNA-dependent RNA polymerases, forming a helical ribonucleoprotein complex (RNP). On the envelope, the viral particle possesses two types of viral glycoprotein spikes, the hemagglutinin (H) and fusion (F) proteins (Griffin, 2007). The H protein is responsible for binding to cellular receptors on the target host cells, and plays a key role in the determination of host cell specificity (tropism) of MV (Yanagi et al., 2009). Binding of the H protein to a receptor triggers F protein-mediated membrane fusion between the virus envelope and the host cell plasma membrane, releasing the RNP into the cytoplasm. In cells infected with MV, the H and F proteins are expressed on the cell surface and cause cell-to-cell fusion, producing syncytia.

DISCOVERIES OF CELLULAR RECEPTORS FOR MV

Basically, MV specifically infects cells expressing its receptors. Therefore, the distribution pattern of its receptors is a key determinant of which cells become infected with MV (Yanagi et al., 2009). The initial discovery of an MV receptor came in 1993 (Dorig et al., 1993; Naniche et al., 1993). Two independent studies indicated that the receptor molecule for MV is the human membrane cofactor protein (MCP/CD46), a central component of the complement system, which is expressed ubiquitously on all organs and tissues throughout the human body (Dorig et al., 1993; Naniche et al., 1993). These findings were highly welcomed from the viewpoint that MV causes a systemic infection. Meanwhile, Kobune et al. (1990) reported the isolation of lymphotropic MV strains, and subsequent studies indicated that these lymphotropic MV strains do not use MCP/CD46 as a receptor (Yanagi et al., 2009). Importantly, Kobune's isolates exhibited a high virulence in experimentally infected monkeys, whereas MCP/CD46-using classical MV isolates caused no or mild disease in monkeys (Kobune et al., 1990, 1996; Takeda et al., 1998). Hence, new two questions have arisen for MV researchers. What is the receptor for these lymphotropic strains? Which strains are the real wild-type (wt) MV strains? In 2000, using Kobune's isolates, another receptor was identified (Tatsuo et al., 2000). This receptor is signaling lymphocyte activation molecule, also known as CD150 (SLAM/CD150), which is expressed on cells of the immune system (Tatsuo et al., 2000). Subsequent studies clarified

that SLAM/CD150 is a receptor for wt MV strains circulating in patients, and that MCP/CD46 does not act as a receptor for wt MV strains (Yanagi et al., 2009). MCP/CD46 acts as a receptor only for vaccine and some laboratory MV strains (Yanagi et al., 2009). Currently, it is clear that these MV strains have acquired the ability to use MCP/CD46 as an alternative receptor to grow in laboratory cell lines lacking SLAM/CD150 expression (Yanagi et al., 2009).

Hence, it has become generally accepted that wt MV is a lymphotropic virus that specifically targets immune cells, similar to the case of human immunodeficiency virus (HIV) and human T cell lymphotropic virus type 1 (HTLV1). In 2000, a recombinant MV, IC323, was generated based on Kobune's first isolate (Takeda et al., 2000), and has greatly contributed to our understanding of the molecular bases for the pathogenesis of wt MV strains (Takeuchi et al., 2005; de Swart et al., 2007; Devaux et al., 2008, 2011; Leonard et al., 2008; Nakatsu et al., 2008; de Vries et al., 2010; Ludlow et al., 2010; Mühlebach et al., 2011; Noyce et al., 2011). At that time, only SLAM/CD150-positive cells were found to be susceptible to wt MV infections. However, it remained difficult to make a final conclusion that SLAM/CD150 is the sole receptor for wt MV, because histopathological examinations of measles patients and monkeys infected with MV have revealed considerable levels of MV protein expression in the epithelia of various organs, and histopathological changes are also evident in these epithelia (Nii et al., 1964; Lightwood and Nolan, 1970; Olding-Stenkvist and Bjorvatn, 1976; Moench et al., 1988; Craighead, 2000; **Figure 1**). In 2003, primary cultures of human small airway epithelial cells (SAECs) were shown to be susceptible to wt MV infection (Takeuchi et al., 2003). Upon MV infection, large syncytia developed in SAECs via a SLAM-independent mechanism (Takeuchi et al., 2003). After searching many cell lines, several epithelial cell lines with high susceptibility to MV infection were identified (Takeda et al., 2007; Tahara et al., 2008). Tahara et al. (2008) and Leonard et al. (2008) clearly demonstrated that wt MV infects epithelial cell lines that form tight junctions (TJs) using an unidentified receptor (Leonard et al., 2008; Tahara et al., 2008). Using these cells and recombinant IC323 expressing green fluorescent protein (IC323-EGFP; Takeda et al., 2000; Hashimoto et al., 2002), a final answer was obtained for the receptor on epithelial cells. Two groups independently demonstrated that nectin4, which is expressed at adherens junctions (AJs), acts as a receptor for MV (Mühlebach et al., 2011; Noyce et al., 2011). Interestingly, MV loses the ability to use either SLAM/CD150 or nectin4 when it possesses specific mutations in the H protein (Leonard et al., 2008; Tahara et al., 2008; **Figure 2**). Nectin4-blind MV still infects SLAM/CD150-positive immune cells efficiently (SLAM/CD150-tropic), and conversely, SLAM/CD150-blind MV infects nectin4-positive epithelial cells efficiently (nectin4-tropic; Leonard et al., 2008; Tahara et al., 2008; **Figure 2**). In this regard, MV is intrinsically dual-tropic to immune cells and epithelial cells (**Figure 2**).

There is now no doubt that SLAM/CD150 and nectin4 are the major receptors for MV. However, other molecules may further support MV infection *in vivo*, being involved in the development of measles and its neurological sequela. For example, the mechanism that the C-type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) acts as an attachment receptor for MV, thereby promoting MV infection of DCs, may be ideal to understand the extraordinarily high transmissibility of measles (de Witte et al., 2006, 2008). It is well known that MV causes subacute sclerosing panencephalitis (SSPE), a persistent infection of the central nervous system (CNS) with MV. This occurs with a mean latency period of 7–10 years after suffering from acute measles at a frequency of 1/5,000–1/100,000 reported cases of acute measles (Takasu et al., 2003; Bellini et al., 2005). The mechanisms underlying the spread of MV in the CNS remain to be elucidated. Although nectin4 is a possible candidate for an MV receptor in the CNS, no (or undetectable) nectin4 expression was observed in the CNS in humans (Reymond et al., 2001; Brancati et al., 2010), and some MV strains derived from SSPE patients are likely to use nectin4 inefficiently (Seki et al., 2011). Data reported by Makhortova et al. (2007) suggest that neurokinin-1, a substance P receptor, supports trans-synaptic transmission of MV by acting as a receptor for the F protein.

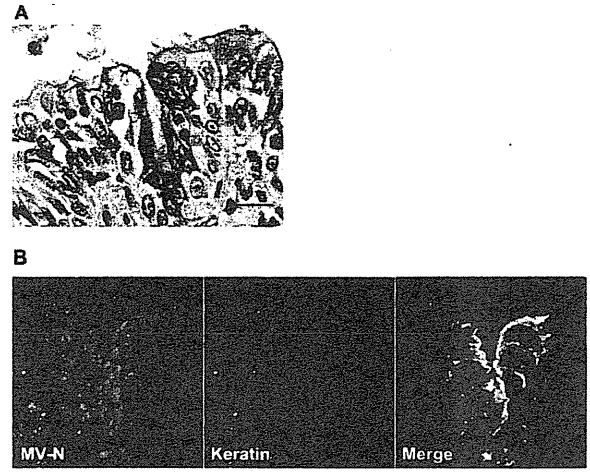

FIGURE 1 | Measles virus antigen and histopathological changes in bronchiolar epithelial cells. At 11 days post-infection, a cynomolgus monkey experimentally infected with wt MV IC323 strain was subjected to the histopathological examination. **(A)** Hematoxylin and eosin staining. Multinucleated cell were seen in the epithelial layer of the bronchiole with inflammatory infiltration of eosinophils and lymphocytes. The multinucleated cells contain eosinophilic intranuclear inclusions. Scale bar indicates 20μm. **(B)** Immunohistochemical double staining for the N protein of MV (left, green) and keratin (center, red). Multinucleated epithelial giant cells showing positive staining for the N protein are present in the bronchiole (right, yellow in merged images). Accumulation of N proteins is detected on the apical surface of ciliated epithelial cells.

SLAM/CD150

Measles virus infection causes immunosuppression in patients and is often accompanied by secondary bacterial infections. Typically, MV-induced immunosuppression is characterized by a marked lymphopenia, and an early T_H1 response followed by predominant and prolonged T_H2 response in patients, with suppression of mitogen-induced lymphocyte proliferation *ex vivo* (Griffin and

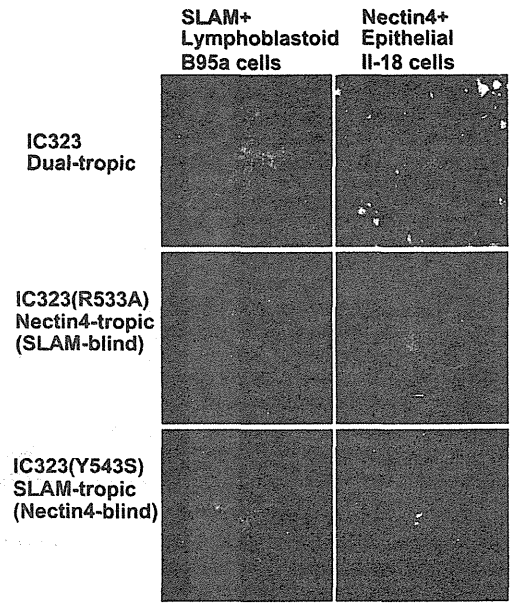

FIGURE 2 | Dual-tropic nature of wt MV. Wt MV (IC323) infects and produces syncytia in both SLAM-positive lymphoblastoid B95a and nectin4-positive epithelial II-18 cells (dual-tropic). IC323 with an R533A mutation in the H protein fails to spread in B95a cells (SLAM-blind), but replicates well in II-18 cells (nectin4-tropic). On the contrary, IC323 with a Y543S mutation in the H protein spreads in B95a cells (SLAM-tropic), but fails to spread in II-18 cells (nectin4-blind). All MVs in this figure are engineered to express EGFP.

Ward, 1993; Schneider-Schaulies and Schneider-Schaulies, 2009). Some, if not all, of these immunological observations must be attributed either directly or indirectly to the fact that MV uses SLAM/CD150 as a receptor. SLAM/CD150 is a member of the SLAM-family receptors, which belong to the immunoglobulin (Ig) superfamily (Veillette, 2010; Ma and Deenick, 2011). The SLAM-family consists of nine members (Cannons et al., 2011; Ma and Deenick, 2011). The SLAM-family receptors are type I transmembrane proteins that typically possess an extracellular region with two Ig-like domains (an amino-terminal variable (V)-like domain and a carboxy-terminal constant-2 (C2)-like domain), a transmembrane region, and a cytoplasmic region that harbors multiple tyrosine-based motifs (Detre et al., 2010; Veillette, 2010; Cannons et al., 2011; Ma and Deenick, 2011). These motifs are referred to as immunoreceptor tyrosine-based switch motifs (ITSMs; Cannons et al., 2011). The SLAM-family receptors are expressed in a broad range of immune cells and play critical roles in immunity. In general, the receptors act as self-ligands and their homophilic *trans*-interactions occur between either heterotypic or homotypic immune cells (Veillette, 2010; Ma and Deenick, 2011). SLAM/CD150 is expressed on thymocytes, subsets of B and T lymphocytes, mature dendritic cells (DCs), macrophages, and platelets, and their expression is upregulated or induced in lymphocytes and monocytes upon activation (Detre et al., 2010; Veillette, 2010; Cannons et al., 2011; Ma and Deenick, 2011).

Signaling lymphocyte activation molecule-associated protein (SAP)-family adaptors [SAP, Ewing's sarcoma-associated transcript (EAT)-2, and EAT-2-related transducer (ERT)] play important roles for the signal transductions mediated by the SLAM-family receptors (Veillette, 2010; Ma and Deenick, 2011). They are small proteins that consist of a single Src homology 2 (SH2) domain and a short carboxy-terminal region. SAP associates intracellularly with the ITSMs in the cytoplasmic region of the SLAM-family receptors via the SH2 domain (Dong and Veillette, 2010; Veillette, 2010; Ma and Deenick, 2011). SAP has the ability to bind concomitantly to the Src-family protein tyrosine kinase, Fyn, thereby coupling the SLAM-family receptors with Fyn (Dong and Veillette, 2010; Veillette, 2010; Cannons et al., 2011). Thereafter, Fyn phosphorylates tyrosine residues at the cytoplasmic region of SLAM-family receptors and other intracellular effector molecules, activating the downstream signals (Detre et al., 2010; Dong and Veillette, 2010; Cannons et al., 2011; Ma and Deenick, 2011). In another mechanism, SAP binding to the SH2 domain of SLAM-family receptors competes with the binding of other SH2 domain-containing molecules, thus modulating the SLAM-mediated signaling (Dong and Veillette, 2010; Veillette, 2010; Cannons et al., 2011). In $CD4^+$ T cells, signals via SLAM/CD150-SAP–Fyn interactions play important roles in regulating T cell receptor-mediated induction of T_H2 cytokines, such as interleukin (IL)-4 and IL-13 (Detre et al., 2010; Cannons et al., 2011; Ma and Deenick, 2011). EAT-2 also mediates the signal transduction cascades of the SLAM-family receptors via a similar but distinct mechanism to that of SAP (Cannons et al., 2011). Similar to SAP, EAT-2 also associates with the ITSMs of SLAM-family receptors through its SH2 domain, but mediates the subsequent signal cascades via its own phosphorylated tyrosine in the short carboxy-terminal region (Veillette, 2010). In general, the signals mediated by the SAP-family adaptors induce the activation and differentiation of immune cells (Veillette, 2010). However, if the SAP-family adaptors are absent, the SLAM-family receptors mediate inhibitory signals to immune cells (a switch-of-function effect; Dong and Veillette, 2010; Veillette, 2010).

Roles for SLAM/CD150 in macrophage functions, cell adhesion, and NKT cell development have also been demonstrated, although many data were obtained in mice (Dong and Veillette, 2010; Veillette, 2010; Cannons et al., 2011; Ma and Deenick, 2011). X-linked lymphoproliferative syndrome is a rare immunodeficiency disease typically caused by mutations in the SAP-encoding gene, *SH2D1A* (Veillette, 2010; Ma and Deenick, 2011). Patients with this syndrome have various functional defects and impaired differentiation of immune cells, indicating crucial roles for SAP in normal immunity (Dong and Veillette, 2010). Hypogammaglobulinemia, massive lymphoproliferative syndrome, and a fatal response to Epstein–Barr virus infection are characteristics of the disease (Veillette, 2010; Ma and Deenick, 2011). Although SLAM-family receptors have some functional redundancy, each receptor plays specific roles in a variety of immune responses (Dong and Veillette, 2010; Veillette, 2010).

NECTIN4

In general, lymphotropic viruses, such as HIV and HTLV1, can never be airborne, and are transmitted inefficiently even through

direct contact with patients. In sharp contrast, MV transmits via aerosols, and has a highly contagious nature. Therefore, this transmission style of MV cannot be easily explained by the fact that the virus uses a lymphocytic molecule, SLAM/CD150, as a receptor. The recent findings showing that MV uses nectin4, a cell adhesion molecule (CAM) expressed at the AJs of epithelia, may partly but nicely explain how and why MV transmits efficiently from a patient to other individuals. Epithelial cells are connected with one another through the formation of several specialized cell–cell junctions, such as TJs, AJs, desmosomes, and gap junctions. TJs function as a physical barrier that prevents the passage of soluble molecules through the intercellular gaps, and also blocks the lateral movement of lipids and membrane proteins across the TJ barrier, thereby acting as the border of the apical and basolateral membranes. AJs are located near the basolateral side of the TJs. They are basically formed by cadherins and nectins and intracellularly connected by actin filaments. Nectin4 is a member of the nectin family, which consists of four members (nectin1, 2, 3, and 4; Takai et al., 2008a). Nectin1 and nectin2 were originally identified as poliovirus receptor-related protein (PRR)-1 and PRR-2, respectively, and subsequently shown to support the entry of some herpes viruses (Takai et al., 2008a,b). Similar to the SLAM-family members, nectins are also type I transmembrane proteins that belong to the Ig superfamily (Takai et al., 2008a,b). In general, they possess an extracellular region with three Ig-like domains (an amino-terminal V-like domain and two C-like domains), a transmembrane region, and a cytoplasmic region with a short afadin-binding motif (Takai et al., 2008a,b). The consensus motif was reported to be E/A-X-Y-V for nectin1, 2, and 3, while nectin4 does not have this motif but still binds to afadin (Reymond et al., 2001; Takai et al., 2008a). Reymond et al. (2001) proposed a new consensus motif, K/R-X-X-Y/L-V, for all four nectins. Afadin is an actin filament (F-actin)-binding protein, and supports nectins to interact and co-operate with cadherins, other CAMs, and intracellular signaling molecules (Takai et al., 2008a).

Nectins are expressed as dimers, and interact *in trans* with other nectin dimers expressed on neighboring cells (Takai et al., 2008a,b). All nectins show homophilic interactions, while heterophilic interactions are also observed between specific nectins, such as those between nectin1/nectin3 and nectin 2/nectin3 (Takai et al., 2008a,b). Some nectin-like molecules also interact with nectins (Takai et al., 2008a,b). Nectin4 shows homophilic interactions as well as heterophilic interactions with nectin1 (Reymond et al., 2001; Takai et al., 2008a). The Ig V-like domain is used for the *trans*-interaction (Reymond et al., 2001; Fabre et al., 2002). Nectins play key roles in the initiation of AJ formation, and regulate various physiological functions of epithelial cells, such as contact inhibition of cell movement and proliferation, survival, differentiation, and cell polarization (Takai et al., 2008a,b).

Although nectin1 and nectin2 are expressed in a broad range of tissues, the expression of nectin3 and nectin4 is more specific (Reymond et al., 2001). Reymond et al. (2001) and Brancati et al. (2010) showed that human nectin4 is expressed mainly in the placenta and to lesser extents in the trachea, prostate, lung, and stomach. In addition, Brancati et al. (2010) demonstrated nectin4 expression in human keratinocytes, suprabasal nucleated layers of the epidermis, and non-keratinized structures of hair. Some levels of expression in epithelial cells of the tonsil, oral mucosa, esophagus, and nasopharynx have also been reported (www.proteinatlas.org). Although, in many cases, nectin4 is expressed in low or undetectable levels in normal human tissues, many cancer cells are highly positive for nectin4. Thus, it has been proposed that nectin4 is a new tumor-associated marker (Fabre-Lafay et al., 2007; Takano et al., 2009; Derycke et al., 2010). These observations may provide a rationale for the use of MV as an oncolytic agent (Mühlebach et al., 2011). In humans, mutations in the *PVRL4* gene encoding nectin4 cause ectodermal-dysplasia-syndactyly syndrome (EDSS), in which patients have affected skin and skin appendages, such as hair, teeth, and nails (Brancati et al., 2010; Jelani et al., 2011).

RELEVANCE OF SLAM/CD150 AND NECTIN4 TO MV PATHOGENESIS

The pathology of measles can now be drawn with these two receptors (**Figure 3**). Although nectin4-expressing epithelial cells can be the initial targets of MV, no or very limited infection of epithelia was observed in monkeys experimentally infected with MV at the early days after infection (Ludlow et al., 2010; Lemon et al., 2011). Instead, MV initiates its infection via SLAM-mediated entry into alveolar macrophages and DCs in the lung or respiratory tracts (de Witte et al., 2008; de Vries et al., 2010; Lemon et al., 2011). These infections may allow MV to penetrate into the human body and reach the lymphoid organs or tissues, where SLAM/CD150-expressing cells are abundant (Corry et al., 1984; Lehmann et al., 2001). After initial replication in these lymphoid organs or tissues, MV or MV-infected lymphocytes can easily enter the bloodstream. Subsequently, a dramatic MV infection is observed in all lymphoid organs, such as the spleen, thymus, appendix, tonsil, and lymph nodes, throughout the body (Moench et al., 1988; Kobune et al., 1996; de Swart et al., 2007; de Vries et al., 2010). At the time when the MV infection of lymphoid organs reaches its peak, MV infection of epithelia, such as squamous stratified epithelia of the tongue and buccal mucosa and ciliated epithelia of the trachea, becomes evident (Nii et al., 1964; Olding-Stenkvist and Bjorvatn, 1976; Moench et al., 1988; de Swart et al., 2007). This epithelial infection is probably led by MV-infected immune cells and initiated through the basolateral side, since monkeys infected with MV often show infectious foci in the epithelia with MV-infected lymphoid or myeloid cells in the subepithelial cell layers of the trachea, bronchus, and tongue (de Vries et al., 2010; Ludlow et al., 2010). The H protein expressed on MV-infected immune cells that migrate through the epithelial cell layer likely recognizes nectin4 expressed at AJs, triggering F protein-mediated membrane fusion between the MV-infected immune cells and the target epithelial cells. Mühlebach et al. (2011) demonstrated a correlation between nectin4 expression and MV infection in epithelia *in vivo*. Importantly, MV has a mechanism that further facilitates virus shedding in the airway. In epithelia, MV selectively releases progeny virus particles from the apical membrane to the luminal side of the respiratory tract (Leonard et al., 2008; Tahara et al., 2008). Leonard et al. (2008) showed that MV genetically engineered to use SLAM/CD150, but not nectin4 (nectin4-blind or SLAM/CD150-tropic), does not shed progeny viruses into the respiratory tract,

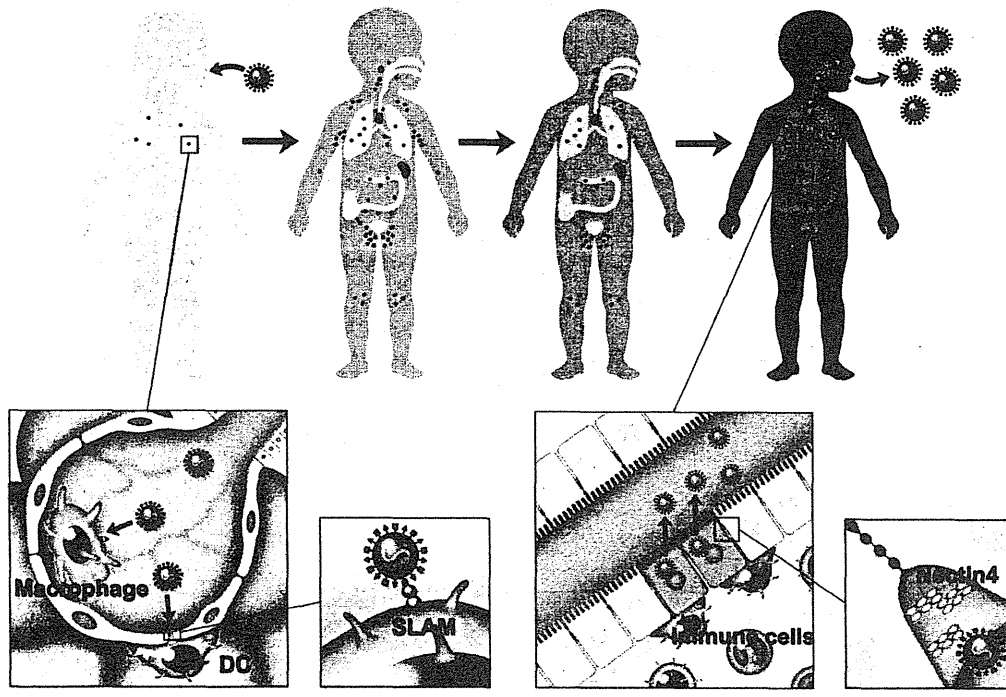

FIGURE 3 | Pathology of measles. MV initiates its infection via SLAM-mediated entry into alveolar macrophages and DCs in the lung. After initial replication in regional lymph nodes, MV enters the bloodstream and subsequently spreads to all lymphoid organs, such as the spleen, thymus, appendix, tonsil, and lymph nodes, throughout the body. When the MV infection of the lymphoid organs reaches its peak, MV-infected immune cells transfer MV to epithelial cells through the basolateral side. At this step, MV uses nectin4 expressed at AJs as a receptor. In the epithelia, MV selectively releases its progeny virus particles from the apical membrane to the luminal side of the respiratory tract.

although it does show systemic infection of lymphoid organs, similar to the case for wt MV.

CONCLUDING REMARKS

Membrane cofactor protein/CD46 was first identified as a receptor for MV (Dorig et al., 1993; Naniche et al., 1993). However, our current knowledge of MV receptors has been totally transformed. In 2000, it was shown that SLAM/CD150 expressed on cells of the immune system, but not MCP/CD46, is a real receptor for wt MV. However, recent studies further showed that SLAM/CD150 is not the sole receptor for MV. MV has an intrinsic ability to enter not only immune cells but also epithelial cells. In 2011, a clear answer was obtained through the identification of the epithelial MV receptor nectin4, which is expressed at AJs, thereby partly explaining why MV exhibits its highly contagious nature (Mühlebach et al., 2011; Noyce et al., 2011). Recent studies on MV receptors greatly advanced our understanding of MV pathogenesis. However, many aspects and molecular mechanisms underlying immunosuppressive effects and a highly contagious nature of MV still remain to be elucidated. Analyses of physiological roles of MV receptors, SLAM/CD150, and nectin4, would provide deep insights into MV pathogenesis.

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