



The potential significance of adaptive evolution and dimerization in chimpanzee intercellular cell adhesion molecules (ICAMs)

Nicole A.R. Walter^{a,1}, Justin Stebbing^b, Walter Messier^{a,*}

^aEvolutionary Genomics, Colorado Bioscience Park Center, 12635 East Montview Boulevard, Aurora, Colorado 80010, USA

^bThe Department of Immunology, Division of Investigative Science, Faculty of Medicine, Imperial College of Science, Technology and Medicine, The Chelsea and Westminster Hospital, London SW10 9NH, UK

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Abstract

Cell adhesion molecules are involved in a diverse array of cellular processes. Recent data suggests that human immunodeficiency virus (HIV-1) co-opts their functions, in particular the properties of the intercellular cell adhesion molecules (ICAMs), to enhance viral infection and transmission. To investigate mechanisms that may underlie the non-progression that occurs in immunodeficiency virus-infected chimpanzees, we amplified the protein coding regions of multiple non-human primate ICAMs 1–5 and two ICAM ligands, leukocyte function-associated antigen-1 (LFA-1) and macrophage antigen 1 (Mac-1). We then employed a phylogenetic tree-based approach to comparative genomics, in order to screen for the presence of adaptive changes. Strong Darwinian positive selection in chimpanzee ICAMs 1, 2 and 3 was observed, most markedly in domains that are critical for the integrity and maintenance of ICAM-1 dimerization. As binding of ligands, including the attachment of virions, is influenced by the state of ICAM 1 dimerization, chimpanzee ICAMs may have evolved to modulate their own dimerization. In concert with previous evidence suggesting an ancient retroviral pandemic as a prominent selective force in chimpanzee evolution, adaptation of chimpanzee ICAMs may have effected a mechanism that explains the lack of immunosuppression observed following HIV-1 or simian immunodeficiency virus (SIV_{cpz}) infection.

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1. Introduction

Humans and our closest living relatives, the chimpanzees, share genomes with high degrees of similarity. However, conspicuous differences exist in how these species respond to pathogens, including hepatitis viruses (Dienes et al., 1990; Maynard et al., 1972; Stebbing et al., 2004), *Plasmodium falciparum* (Ollomo et al., 1997) and other viruses such as rhinoviruses and most notably,

HIV-1 (Hahn et al., 2000). It has long been recognized that common chimpanzees (*Pan troglodytes*), although occasionally infected by SIV and susceptible to infection by HIV-1, are generally resistant to progressive immunosuppression (Hahn et al., 2000; Santiago et al., 2002). The demonstration that the progenitor of HIV-1, SIV_{cpz} originated in chimpanzees (Gao et al., 1999) suggests that their resistance may stem from evolutionary accommodation by chimpanzees to infection by this CD4 tropic lentivirus (Hahn et al., 2000; Weiss and Wrangham, 1999).

A previous study has revealed that chimpanzees have lost many major histocompatibility complex (MHC) class I A, B, and C loci (de Groot et al., 2002) and that this depletion occurred before known divergence times of modern chimpanzee subspecies. In turn, these data

*Corresponding author. Tel.: +1 720 859 4084; fax: +1 720 859 4076.

E-mail addresses: waltern@ohsu.edu (N.A.R. Walter), j.stebbing@imperial.ac.uk (J. Stebbing), wmessier@evolgen.com (W. Messier).

¹Present Address: Behavioral Neuroscience, Oregon Health and Science University, Portland, Oregon 97239, USA.

have indicated that a selective sweep in the MHC class I gene may be a result of a previous viral epidemic (de Groot et al., 2002). Indeed, modern chimpanzee populations most likely represent the descendants of AIDS-resistant animals, who survived such an ancient HIV-like pandemic (de Groot et al., 2002).

The evolution of complex organisms has been dependent on the capacity of their constituent cells to communicate with each other and the external environment (Bockaert and Pin, 1999). Recently a dendritic cell (DC) specific cell surface lectin was identified (Geijtenbeek et al., 2000a, b) that captures carbohydrate moieties on the coat of HIV-1. This lectin, referred to as DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), mediates clustering of DC with T lymphocytes (Geijtenbeek et al., 2000a, b). It has been shown that HIV-1 can be propagated between cells by such molecules either in *cis*, by concentration of the virus on target cells, or in *trans* (Kwon et al., 2002), by transmission of bound HIV-1 to a target cell expressing the appropriate entry receptors, CD4 and either CXCR4 or CCR5. McDonald et al. (2003) have recently suggested that HIV-1 has usurped the antigen capture abilities of DCs, which promotes the infection of CD4+ T cells in vivo, where the hallmark of HIV infection is loss of CD4+ T cells.

However, while DC-SIGN captures, protects and transmits HIV-1, it does not mediate the cell–cell contact that appears critical in HIV-1 pathogenesis. Such cell–cell contact is mediated by the intercellular adhesion molecules (ICAMs). Several roles these DC-SIGN ligands in progression to AIDS in HIV-1-infected humans have been detailed. For example, the presence of the ICAM ligand leukocyte function-associated antigen-1 (LFA-1) on T cells promotes both HIV adsorption and infectivity (Fortin et al., 1998) and it is thought that interactions between ICAM 1, exposed at the surface of the virus, and LFA-1 expressed on T cells, enhances HIV attachment (Bobardt et al., 2003). Supporting this, HIV-1 replication is decreased by reagents that interrupt interactions between ICAM 1 and LFA-1 (Bounou et al., 2002).

Further, the selective incorporation of host-derived ICAMs into the HIV virion coat results in increased viral infectivity in vitro, a process also dependent on the ICAM/LFA-1 interaction (Fortin et al., 1997). There is also evidence suggesting that this increased infectivity may extend to cells that are not considered normally permissive to HIV-1 (Castilletti et al., 1995). In addition, the recently advanced “Trojan exosome” hypothesis (Gould et al., 2003) postulates that HIV, and in fact, all retroviruses, exploit the cellular exosome exchange pathway to achieve infection, while avoiding detection by the host’s adaptive immune responses. Importantly, ICAMs and the ICAM ligand DC-SIGN (Hwang et al., 2003) are known to play a role in cellular exosome exchange.

We have therefore employed comparative genomics to study adaptive changes within the ICAM family. We present here evidence for the action of intense positive selection (i.e. adaptive evolution) on the chimpanzee ICAMs 1, 2, and 3. This selective pressure most likely resulted from the introduction of an ancestral immunodeficiency virus or related retrovirus into chimpanzee populations. As functional effects mediated by ICAMs depend in part upon their interaction with the ligands LFA-1 and Mac-1, we also carefully examined the ICAM ligands LFA-1 and Mac-1 (but found no evidence for the action of positive selection on these proteins in chimpanzees).

Finally, we postulate that adaptive changes within ICAMs have conferred resistance to immunodeficiency in the chimpanzee by altering the milieu of cell–cell contact between cells.

2. Results and discussion

2.1. Molecular-level adaptation of chimpanzee ICAM protein coding regions

The data herein, which support the adaptive evolution of chimpanzee ICAMs 1, 2, and 3, the previous evidence that chimpanzees sustained a major evolutionary ‘bottle-necking event’ as a result of the introduction of the ancestral immunodeficiency virus, and the roles that these cell adhesion proteins play in progression to AIDS (discussed below, with references), implicate chimpanzee ICAMs in the protection against disease progression observed following HIV-1-infection of chimpanzees.

ICAM genes were sequenced from both human and non-human primates. Using a comparative evolutionary sequence analysis, we found that ICAM genes 1–3 have been subjected to molecular-level Darwinian positive selection (Table 1). Pairwise comparisons of the protein-coding regions of the chimpanzee and human ICAM genes yield statistically significant elevations of their K_a/K_s ratios (Table 1). K_a/K_s values > 1 strongly suggest the action of molecular-level positive selection, i.e. Darwinian adaptation (Messier and Stewart, 1997; Nei, 1987).

As more than 99% of all mammalian genes studied do not display elevated K_a/K_s ratios (Kreitman and Akashi, 1995), and those that do rarely display ratios of the magnitude seen in Table 1 (Kreitman and Akashi, 1995; Messier and Stewart, 1997), an intense selective event most likely explains the results observed.

While pairwise comparisons can detect the action of positive selection, such comparisons alone do not reveal which of the two genes compared (i.e. chimpanzee or human) has been positively selected. A phylogenetic, tree-based analysis can accomplish this (Messier and Stewart, 1997) and we thus applied a phylogenetic

Table 1

 K_a/K_s ratios, and values of K_a and K_s for pairwise comparisons between primate ICAM genes (a) ICAM 3. (b) ICAM 2. (c) ICAM 1

	Chimpanzee 1	Chimpanzee 2	Human 1	Human 2	Gorilla	Orangutan	Rhesus
(a) ICAM 3							
Chimpanzee 1	—	Div/0	2.2	Div/0	2.3	0.71	0.63
Chimpanzee 2	0.080, 0.00	—	2.0	Div/0	2.1	0.69	0.62
Human 1	0.76, 0.34	0.68, 0.34	—	0.30	1.3	0.64	0.57
Human 2	0.67, 0.00	0.58, 0.00	0.10, 0.34	—	2.2	0.67	0.58
Gorilla	0.81, 0.35	0.73, 0.35	0.89, 0.70	0.78, 0.35	—	0.69	0.59
Orangutan	3.2, 4.5	3.1, 4.5	3.1, 4.9	3.0, 4.5	3.1, 4.5	—	0.51
Rhesus	5.6, 8.9	5.5, 8.9	5.5, 9.6	5.4, 9.2	5.7, 9.6	5.0, 9.9	—
	Chimpanzee 1/2	Human 1	Human 2	Gorilla	Rhesus		
(b) ICAM 2							
Chimpanzee 1/2	—	Div/0	Div/0	0.95	1		
Human 1	0.57, 0.00	—	Div/0	0.64	0.96		
Human 2	0.78, 0.00	0.20, 0.00	—	0.76	0.93		
Gorilla	1.8, 1.9	1.2, 1.9	1.4, 1.9	—	0.91		
Rhesus	10.0, 9.8	9.3, 9.8	9.1, 9.8	9.3, 10.0	—		
	Chimpanzee	Bonobo	Human 1	Human 2	Gorilla	Orangutan	Rhesus
(c) ICAM 1							
Chimpanzee	—	0.58	2.2	2.1	1.3	1.0	0.75
Bonobo	0.10, 0.18	—	1.9	1.8	1.2	1.0	0.72
Human 1	2.0, 0.91	2.1, 1.1	—	Div/0	1.1	0.89	0.75
Human 2	1.9, 0.91	2.0, 1.1	0.082, 0.00	—	1.1	0.87	0.74
Gorilla	2.6, 2.0	2.8, 2.2	2.3, 2.1	2.2, 2.1	—	0.95	0.75
Orangutan	5.0, 4.8	5.0, 5.0	4.3, 4.8	4.2, 4.8	4.8, 5.1	—	0.72
Rhesus	7.3, 9.8	7.2, 10.0	7.2, 9.6	7.1, 9.6	7.5, 10.0	6.9, 9.6	—

K_a/K_s ratios are shown above the diagonal; values of K_a and K_s are below, in the format K_a, K_s . All values of K_a and K_s are shown 100X actual values for clarity. All values were calculated with the computer program Li93. Statistically significant comparisons are shown in **bold**, with levels of statistical significance indicated by font style as follows. $P < 0.05$, $P < 0.01$, $P < 0.005$. The only comparisons that display a K_a/K_s ratio > 1 , and are also statistically significant, are those that include a chimpanzee ICAM sequence. It should be noted that while a minority of non-chimpanzee comparisons show $K_a/K_s > 1$, these are not statistically significant, and must therefore be discounted. As such comparisons are subject in some measure to stochastic variation, standards of the field accept only those comparisons with statistical support as providing evidence of positive selection. Note that in those cases when $K_s = 0$, if K_a is significantly greater than K_s , then positive selection can still be inferred for that comparison. Two different chimpanzees and two different human individuals were sequenced. For each of the three ICAM genes, where sequences were found to differ between individuals, these are listed separately; if both individuals had identical sequences, they are listed together on one line with a slash (/) between them. All pairwise comparisons that involve rhesus ICAM 3 sequences show $K_a/K_s < 1$, often with statistical significance. Although it is possible that these comparisons may demonstrate the role of negative selection, it is also possible that such pairwise comparisons are too evolutionarily distant, so that K_s overtakes K_a .

tree-based test, the bN/bS test (Zhang et al., 1998). The results confirmed that for ICAMs 2 and 3, positive selection was likely confined to the chimpanzee lineage (Fig. 1). (Although the K_a/K_s ratio for the human lineages for these genes appears elevated, these comparisons have no statistical support, and must therefore be discounted. As such comparisons are subject in some measure to stochastic variation, standards of the field accept only those comparisons with statistical support as providing evidence of positive selection.)

While the pairwise comparisons show that ICAM 1 has been positively selected between chimpanzees and humans, the resolution of the bN/bS test is insufficient for the ICAM 1 data set to definitively assign positive selection to either the chimpanzee or human lineage. For ICAM 1, we thus further analysed our dataset using

outgroup analysis, an alternative approach that considers between-species pairwise comparisons in phylogenetic context. This analysis suggests that positive selection on ICAM 1 was also confined to the chimpanzee lineage (The very recent divergence of the *Homo/Pan* lineages prevents *absolute* assignment of positive selection exclusively to the chimpanzee lineage; this does affect our hypothesis that adaptation to an immunodeficiency virus as the selective pressure responsible for these exceptional K_a/K_s ratios. Clearly, only subsequent biochemical validation can definitely confirm this hypothesis.)

Although it remains formally possible that elevated K_a/K_s ratios may reflect a locus or chromosomal-specific anomaly (such as suppression of K_s due to isochoric differences in GC content) rather than the effects of

positive selection; this appears most unlikely in the present case. First, K_s values for the primate ICAM 1, 2, and 3 genes were compared to those of previously estimated other well-studied loci, including the primate lysozymes and the non-coding β -globin loci and we observed no evidence for a significant difference in these values. Second, examination of the GC content of the primate ICAM 1, 2, and 3 genes shows no significant

differences from mean mammalian values (data not shown). Finally, K_a/K_s values of the functionally distinct remaining two members of the ICAM gene family, ICAMs 4 and 5, were examined, and showed no evidence for positive selection. Like the overwhelming majority of genes, which are not positively selected (Kreitman and Akashi, 1995; Messier and Stewart, 1997), ICAMs 4 and 5 display K_a/K_s ratios < 1 (ICAM 4, chimpanzee-to-human: $K_a/K_s = 0.51$, ICAM 5, chimpanzee-to-human: $K_a/K_s = 0.15$). Thus ICAMs 4 and 5 both appear to be well conserved between humans and chimpanzees. Interestingly, ICAM 5 maps only 5 kb away from ICAM 1 on chromosome 19 (Kilgannon et al., 1998), further supporting our data that the elevated K_a/K_s ratio displayed by comparison of human and chimpanzee ICAM 1 is not the result of a locus or chromosomal anomaly.

2.2. The role of cell-cell adhesion proteins in HIV infection and disease progression

Cell-cell contact appears critical in many diseases, in particular, in the dissemination, propagation and transmission of HIV-1 within an individual (McDonald et al., 2003). The ICAM proteins mediate such cell-cell contact, and several roles for ICAMs in progression to AIDS in HIV-1-infected humans have been previously suggested. The presence of the ICAM ligand leukocyte function-associated antigen-1 (LFA-1) on T cells promotes both HIV adsorption and infectivity (Fortin et al., 1998) and it is thought that interactions between ICAM 1, exposed at the surface of the virus, and LFA-1 expressed on T cells, enhances HIV attachment (Bordard et al., 2003). Supporting this, HIV-1 replication is decreased by reagents that interrupt interactions between ICAM 1 and LFA-1 (Bounou et al., 2002).

The selective incorporation of host-derived ICAMs into the HIV virion coat results in increased viral infectivity in vitro, a process also dependent on the ICAM/LFA-1 interaction (Fortin et al., 1997). There is also evidence suggesting that this increased infectivity

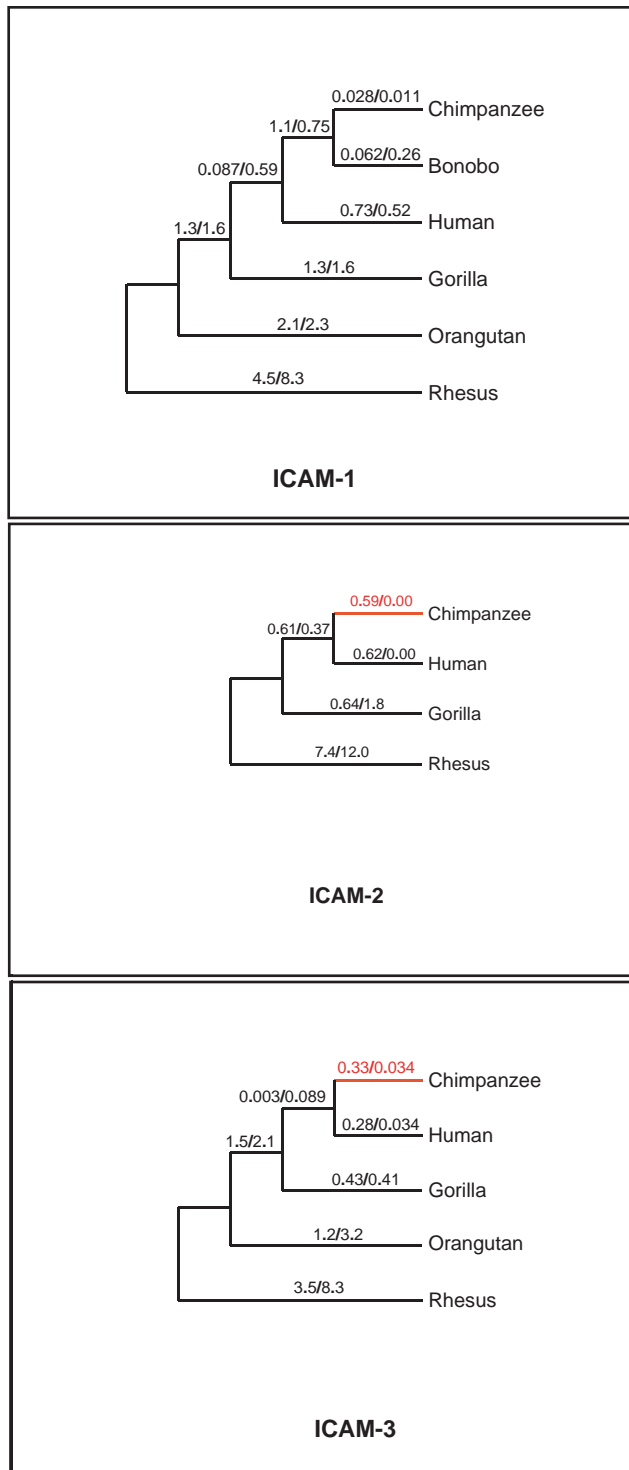


Fig. 1. Results of bN/bS test for assigning molecular positive selection to lineages bN/bS estimates were calculated using the “bN/bS” program, for ICAMs 1, 2, and 3, and are shown mapped onto the appropriate lineages of the accepted phylogeny for these species. All values are shown 100X for clarity. Statistically significant comparisons are shown in red. The bN/bS test assigns positive selection to ICAMs 2 and 3 unambiguously on the chimpanzee lineage; resolution of the test is insufficient for ICAM 1, although outgroup analysis confirms that positive selection on ICAM 1 was limited to the chimpanzee lineage. Note that there is some difference in the species from which ICAMs 1, 2, and 3 were sequenced. In order to make application of the bN/bS test as conservative as possible, we show only those individuals for which bN values are lowest, and bS values are highest. Thus we used the human 1 sequence (as designated in Table 1) for ICAMs 1 and 2; human 2 and chimpanzee sequence 2 were used for ICAM 3.

may extend to cells that are not considered normally permissive to HIV-1 (Castilletti et al., 1995). In addition, the recently advanced “Trojan exosome” hypothesis (Gould et al., 2003) postulates that HIV, and in fact, all retroviruses, exploit the cellular exosome exchange pathway to achieve infection, while avoiding detection by the host’s adaptive immune responses. Importantly, ICAMs and the ICAM ligand DC-SIGN (Hwang et al., 2003) are known to play a role in cellular exosome exchange.

2.3. Structure/function implications of changes in chimpanzee ICAMs

To understand the physiological impacts of adaptive changes on ICAM interactions, we analysed key ICAM 1 dimerization and binding regions and examined compensatory or adaptive changes in the ICAM 1 ligands LFA-1 and macrophage antigen 1 (Mac-1). ICAM 1 is present on the cell surface as a dimer and such dimerization facilitates binding of ICAM 1 with LFA-1 (Jun et al., 2001a). ICAM 1 domains 1 and 2 are immunoglobulin-like domains that contain the binding site for LFA-1 (Staunton et al., 1990) and also play a significant role in ICAM 1 dimerization (Casasnovas et al., 1998; Jun et al., 2001a). Our sliding window K_a/K_s analyses revealed that the portions of the ICAM 1 gene that display the highest K_a/K_s ratios in human/chimpanzee comparisons were those that code for domains 1 and 2 ($K_a/K_s = 3.1$, $P < 0.5$, versus $K_a/K_s = 2.0$ for the entire ICAM 1 coding sequence). By contrast, analysis of domain 3, which does not play a role in either ICAM 1 dimerization (Jun et al., 2001a, b), or LFA-1 binding (Jun et al., 2001a, b; Staunton et al., 1990), suggests evolutionary conservation (K_a/K_s ratio = 0.60).

Next, using crystal structures, the locations of the unique chimpanzee amino acid replacements in ICAM 1 were examined, with respect to amino acids that are critical for binding and dimerization (Bella et al., 1998; Casasnovas et al., 1998). In Fig. 2 we indicate the unique chimpanzee amino acid replacements (those replacements that have occurred in chimpanzee ICAM 1, but not in any of the other primate ICAMs, including human) and also the amino acids important for ICAM 1 dimerization and for binding to LFA-1.

One of the amino acid replacements unique to the chimpanzee lineage, Leu-18 to Gln-18, is located in the area critical for human ICAM 1 dimerization (Jun et al., 2001b) a domain that contains a leucine cluster to create a hydrophobic dimerization surface (Jun et al., 2001b). Our sequencing results show that in chimpanzee ICAM 1, one of these leucines is replaced by the more hydrophilic glutamine (see Fig. 2; hydrophobicity scores 3.8 Leu to -3.5 Gln). The distortion of the hydrophobic surface in chimpanzee ICAM 1 suggests that selective

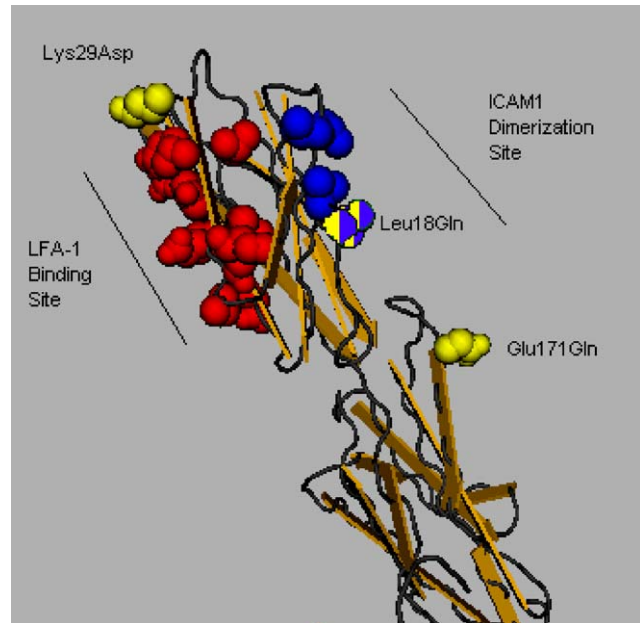


Fig. 2. Cartoon of the crystal structure of dimerized domains 1 and 2 of ICAM 1. Amino acid replacements that are unique to the chimpanzee ICAM 1 lineage are labeled and shown in space-filling mode, in yellow. Residues known to be critical for ICAM 1/LFA-1 binding are shown in space-filling mode, in red. Residues known to be critical for ICAM 1 dimerization are shown in space-filling mode, in blue. Residue 18, a critical residue for ICAM 1 dimerization, is also a unique amino acid replacement in chimpanzee ICAM 1, thus is shown as both yellow and blue. Also labeled are clusters of residues that form parts of the LFA-1 binding site, and the ICAM 1 dimerization site. Unique chimpanzee amino acid replacements: Leu18Gln, Lys29Asp, Glu171Gln (nomenclature: human residue, residue number, chimpanzee residue). Critical ICAM 1/LFA-1 binding residues: Leu-30, Leu-31, Ile-33, Glu-34, Thr-35, Pro-36, Lys-39, Gln-62, Met-64, Thr-66, Asn-68, Gln-73. Critical dimerization residues: Leu-18 (Gln in chimpanzee), Leu-42, Leu-43, Leu-44. Figure derived from crystal structure in Bella et al. (1998).

pressure may have been directed towards mediating ICAM 1 dimerization in the chimpanzee.

In contrast, all ICAM 1 residues thought to be involved in human LFA-1 binding (Diamond et al., 1991; Edwards et al., 1998; Fisher et al., 1997; Shimaoka et al., 2003) are identical in chimpanzee and human ICAM 1 (See Fig. 2 and Supplementary Data Sections 1 and 2. The Supplementary Data document is available from the senior author, W.M.).² Indeed, these critical residues are highly conserved in all of the primate ICAMs examined (this pattern of extreme conservation is extended to ICAMs 2 and 3: all ICAM 2 residues but one identified as critical for LFA-1 binding are identical between chimpanzee and human; all ICAM 3 residues identified as critical for LFA-1 binding are identical

²Data deposition: The sequences reported in this paper have been deposited in GenBank under the 36 accession numbers AF340033 to AF340068. Supplementary Data are available from the senior author (W.M.) and available at <http://www.evolgen.com>.

between chimpanzee and human (see Supplementary Data Section 2, Footnote 2).

Examination of the chimpanzee LFA-1 deduced protein sequence indicates that residues critical for ICAM 1 binding (Huth et al., 2000; Register et al., 1991) are also identical between chimpanzees and humans. Similarly, all LFA-1 residues known to be critical for binding to ICAMs 2 and 3 are also identical between chimpanzee and human (see Supplementary Data Section 2, footnote 2). Pairwise K_a/K_s comparisons of the chimpanzee and human LFA-1 genes also suggest conservation (The LFA-1 protein contains two subunits, designated α and β : Human LFA-1 α subunit to the chimpanzee LFA-1 α subunit: $K_a/K_s = 0.30$; Human LFA-1 β subunit to the chimpanzee LFA-1 β subunit: $K_a/K_s = 0.053$.) Thus, it is likely that the ICAM 1/LFA-1 binding interaction is fundamentally unchanged between humans and chimpanzees. However, this interaction is known to be indirectly influenced by the state of ICAM 1 dimerization, which, as described above, does appear to have been modulated in the chimpanzee as a result of adaptive evolution.

One unique chimpanzee ICAM 1 replacement, Lys-29 to Asp-29, is immediately adjacent to a cluster of ICAM 1/LFA-1-binding residues, particularly Asn-66, which forms part of the contact surface for ICAM 1/LFA-1 binding (Edwards et al., 1998; Shimaoka et al., 2003). The amide side chain of Asn-66 is known (Edwards et al., 1998) to interact with Glu-241 of LFA-1, an interaction that has been shown to be absolutely critical for ICAM 1/LFA-1 binding (Casasnovas et al., 1998; Edwards et al., 1998; Fisher et al., 1997; Shimaoka et al., 2003). The interaction of Asn-66 with Glu-241 may be influenced by the replacement of the basic Lys-29 (humans) with the acidic Asp-29 (chimpanzee).

Lys-29 is identified as a binding amino acid for the major group of human rhinoviruses, which use human ICAM 1 as a receptor (Register et al., 1991). We considered the possibility that the selective force acting upon chimpanzee ICAM 1 was exposure to the rhinoviruses. However, this seems unlikely as one would have expected evidence of this selective pressure on the many ICAM 1 amino acid residues that are known to be involved in rhinovirus binding. All residues apart from Lys-29 are identical between humans and chimpanzees and remain conserved in all the primate sequences examined.

The most critical residue (Staunton et al., 1990) for binding rhinovirus (Gln-58) is also identical between human and chimpanzee ICAM 1 (see Supplementary Data Section 2, footnote 2) Residue 49 is the only other rhinovirus-binding site that differs between chimpanzee and human; in this case, the chimpanzee sequence retains the ancestral Trp, while human shows a derived Arg, i.e. the human ICAM 1 sequence has changed, while the chimpanzee sequence has been conserved.

Thus, this site provides evidence that exposure to rhinoviruses was not a selective force on chimpanzee ICAM 1.

As noted above, ICAM 1 also binds Mac-1. As for LFA-1, it appears unlikely that the binding interaction of ICAM 1 and Mac-1 has been the target of positive selection between chimpanzees and humans, for three reasons. First, pairwise comparisons of the chimpanzee and human Mac-1 genes suggest conservation (Like LFA-1, Mac-1 contains an α and a β subunit. Human Mac-1 α subunit to the chimpanzee α subunit: $K_a/K_s = 0.30$. Human Mac-1 β subunit to the chimpanzee Mac-1 β subunit, $K_a/K_s = 0.42$). Second, domain 3 of ICAM 1 has long been known to be critical for Mac-1 binding (Diamond et al., 1991). As noted above, unlike domains 1 and 2, this domain is very well conserved between humans and chimpanzee ICAM 1. Third, ICAM 1 residues shown to be critical (Diamond et al., 1991) for Mac-1 binding (Asp-229, Asn-240, Glu-254, Asn-269) are identical between human and chimpanzee ICAM 1; indeed these are almost completely identical in all primate ICAM 1 sequences examined (see Supplementary Data Section 2, see footnote 2).

While de Groot et al. (2002) suggest that chimpanzee resistance to progression to AIDS may result from the limited set of MHC orthologs that modern chimpanzees retain, this explanation we postulate is questionable. First, human populations retain homologs of these same chimpanzee MHC proteins in relatively high frequencies, yet humans, with only very limited exceptions, do not appear naturally resistant to HIV-1-induced immunodeficiency. Second, the analysis presented by de Groot et al. (based upon use of Tajima's " D ", a statistical test for the action of positive selection) suggests that these genes have evolved neutrally. There is no support for positive selection on these chimpanzee loci, although MHC genes in other species have been documented to show molecular level selection (Hughes and Nei, 1988, 1989). Chimpanzee resistance to HIV-1 progression is unlikely to be conferred by the MHC alleles that remain in present day chimpanzee populations.

2.4. Chimpanzee resistance to AIDS may have resulted from adaptive evolution of ICAMs

Our data suggest a model in which positive selection upon the chimpanzee ICAM proteins resulted in chimpanzee resistance to disease progression. As detailed above, the changes seen in chimpanzee ICAM 1, in particular, appear likely to modulate dimerization of chimpanzee ICAM 1. As ICAM 1-mediated cell adhesion functions (such as those exploited by HIV-1) are dependent upon binding to ligand, and as such binding has been shown to be influenced by the state of ICAM 1 dimerization, we propose that binding of chimpanzee

ICAM 1 to its ligands is affected, thus altering the cell adhesion functions needed by HIV-1, perhaps severely reducing viral infectivity. This leads to testable predictions about the differences between dimerization patterns of chimpanzee and human ICAM 1.

Further testable predictions may be postulated regarding chimpanzee ICAMs: they may prevent or retard the increase in cell-to-cell infectivity seen in humans after viral incorporation of host ICAMs, and chimpanzee ICAMs may also prevent or retard DC-SIGN mediated viral dissemination. While ICAMs provide a perfect illustration of F. Jacob's famous idea that 'Evolution is molecular tinkering' (Bockaert and Pin, 1999), further investigation of chimpanzee ICAMs should help define the molecular mechanism that confers resistance to progression of HIV-1-infected chimpanzees, thus delineating potential new strategies to fight HIV/AIDS.

3. Materials and methods

Total RNA was prepared (using the Rapid Total RNA kit, 5'-3', Inc., Boulder, CO) from whole primate bloods. Poly A RNA was isolated from total RNA (using Mini-Oligo dT Cellulose Spin Columns, 5'-3', Inc.) and used as template for the reverse transcription of cDNA (Ready To Go kit, APBiotec, Piscataway, NJ).

The protein-coding region of the primate ICAM genes 1–3 was amplified from cDNA or genomic DNA, using primers (100 ng/ml) designed from the published human sequences (primer sequences available from WM). PCR reactions were carried out using HotStar Master Mix (Qiagen, Valencia, CA) or Taq PCR Core Kit (Qiagen). 20 μ l PCR reactions were run on a nuclease-free agarose gel and purified using gel extraction spin columns (Qiagen). Both strands of the amplification products were sequenced directly (see Supplementary Data Section 3, footnote 2); in the case of human and chimpanzee ICAM 3, PCR products were cloned. PCR products to be cloned were gel purified using a nuclease free agarose gel and Qiagen gel extraction columns and ligated into the pT-Adv vector (Clontech, Palo Alto, CA). The ratio of vector to PCR insert was 1:1. The ligation was then transformed into TOP10F *E. coli* competent cells following the Clontech protocol. Appropriate colonies were removed and grown in 3 ml overnights of Difco Terrific Broth (Detroit, MI). Plasmid DNA was isolated from these overnights using the Qiagen Turbo prep protocol on the Qiagen BioRobot 9600. All sequencing reactions used the Big Dye Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and were then analysed on a 373A DNA sequencer (Applied Biosystems). Independent amplifications were performed, and then sequenced, for each

sequence reported. We also sequenced ICAMs 4 and 5, the genes that code for the chimpanzee (shared) LFA-1 and Mac-1 α subunits, and the LFA-1 and Mac-1 β subunit (the species, number of individuals, and the number of nucleotides sequenced for each gene examined are found in Supplementary Data Section 3, footnote 2).

Appropriate ethical approval was obtained for use of all samples and these were obtained from immunodeficiency virus-free animals.

Values for K_a and K_s were calculated using the computer programs 'Li93' (Li et al., 1985; Li, 1993) and 'bN-bS' (Zhang et al., 1998). Calculated values for K_a and K_s (for the regions of each gene that codes for the mature peptide) were used to determine whether the signal of molecular level Darwinian positive selection was present for ICAMs 1–5, and for the genes coding the ICAM ligands LFA-1 and Mac-1 (GenBank Accession numbers: AF340033 to AF340068 inclusive).

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