Integrin-nucleated Toll-like receptor (TLR) dimerization reveals subcellular targeting of TLRs and distinct mechanisms of TLR4 activation and signaling

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Abstract Toll-like receptors (TLRs) are activated by microbial structures. To investigate the mechanisms of TLR activation, the 10 human TLRs were expressed as chimeras with the integrin αv and β5 subunits. Co-expression of the αv-TLR and β5-TLR chimeras in 293T cells generated 10 TLR homodimers, but only TLR4/4 could effectively activate NF-κB. TLR4 monomers also activated NF-κB but it was enhanced upon homodimerization. The TLR homodimers showed differential surface/intracellular expression. In TLR heterodimers, only TLR2/1 and TLR2/6 were potent in NF-κB activation. NF-κB activation by TLR2/1, TLR2/6 and the TLR4 monomer, but not TLR4/4, was completely inhibited by dominant negative MyD88, suggesting that TLR4 homodimers and monomers could activate NF-κB through different mechanisms.

Key words: Integrin; Toll-like receptor; Dimerization; NF-κB; MyD88; PAMP

1. Introduction

Innate immunity is the first line of host defense against microbial invasion and it relies on pattern recognition receptors (PRRs) to distinguish the different types of pathogens [1,2]. Toll-like receptors (TLRs) are a family of PRRs that specifically recognize different pathogen-associated molecular patterns (PAMPs) [3–5] and potentially function as adjuvant receptors that modulate immune responses according to the type of pathogens [6–8]. The mechanisms by which the different TLRs are activated remain to be determined.

TLRs are type I receptors that contain a large, leucine-rich extracellular domain and a Toll/IL-1R homology (TIR) cytoplasmic domain [9]. IL-1R is activated through heterodimerization or clustering with a related receptor called IL-1R accessory protein (IL-1R AcP) [10–12]. TLR2 activation by certain PAMPs also requires the cooperation of TLR1 or TLR6 [13–15]. When TLR2 was expressed as CD4-nucleated dimers, the TLR2/1 and TLR2/6 heterodimers, but not the TLR2/2 homodimers, were found to activate NF-κB [13]. In fact, TLR2 can also form constitutive heterodimers with TLR1 or TLR6 upon over-expression that activate NF-κB independent of PAMP stimulation [13]. CD4-nucleated TLR4 homodimers also effectively activate NF-κB [13,16,17]. These studies suggested that dimerization may be a common mechanism of TLR activation.

To examine whether other TLRs are also activated through dimerization, we expressed the transmembrane/cytoplasmic (TM/Cyt) domains of all 10 TLRs as chimeras with the extracellular domains of the integrin αv and β5 subunits. Co-expression of the αv-TLR and β5-TLR chimeras generated all possible TLR dimers. NF-κB activation by these TLR dimers was examined.

2. Materials and methods

2.1. Reagents and cell culture

The 293T human embryonic kidney cells were cultured at 37°C in the presence of 5% CO_2, in DMEM supplemented with 10% bovine calf serum (HyClone), 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, and 0.0012% 2-mecaptolethanol. The human monocytic THP-1 cells were cultured in RPMI1640 with the same supplements and were activated for 24 h with lipopolysaccharide (LPS) derived from *Escherichia coli* O55:B5 (Sigma Chemical, St. Louis, MO, USA; 100 ng/ml). RNA was isolated from activated THP-1 cells using the Trizol reagent (Life Technologies). Anti-His_6 (clone BMG-His-1) and anti-c-myc (clone 9E10) were purchased from Roche Molecular Biochemicals. Anti-human integrin β5 (clone B5-IVF2) was obtained from Upstate Biotechnology (Lake Placid, NY, USA). Anti-human integrin αv (clone P3G8) and αvβ5 heterodimer (clone P1F6) were purchased from Chemicon International (Harrow, UK).

2.2. Expression vectors

cDNA encoding the extracellular domains of the integrin αv and β5 subunits were each cloned into the pcDNA3.1 vector. The αv cDNA was amplified by RT-PCR in three fragments using the following primer pairs (5′-3′) designed based on the published αv sequence [18], i.e. tcccggcttggcgtccc/ctatatctgtggctcctttcattg, acgcagtcccatctcaaatcctt/catcttcttcagtctcagggttc and gctcaaatctatttgacaaagtaag/ttgttcgaacacaggcatgggcgctggct. The three PCR fragments were digested with *Hin*dIII, *Hin*dIII/*Xho*I, and *Xho*I/*Sfu*I, respectively, and simultaneously cloned into the *Eco*RV/*Sfu*I site of the pcDNA3.1 vector in frame at the 3′ end with sequences encoding the c-myc and His_6 tags (pαv/MH). A stop codon was introduced between the myc- the His-coding sequences, using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA), to generate the pαv/myc vector. cDNA for the extracellular domain of β5 was amplified using the following primer pair (5′-3′) [19], i.e. tggtggaattcccgcggagtgcagcga/tttttgttcgaagttgggggtgtttccaca, and cloned into the *Eco*RI/*Sfu*I site of the

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Abbreviations: TLR, Toll-like receptor; PAMP, pathogen-associated molecular pattern; Mal, MyD88-adaptor-like protein; GFP, green fluorescence protein; LPS, lipopolysaccharide; PRR, pattern recognition receptor

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pcDNA3.1 vector (pβ5/MH). Sequences encoding the TM/Cyt domains of TLRs were amplified by PCR from the THP-1 cDNA using the following primer pairs (5′-3′): TLR1, cccttcgaaaacataactctgctgatcgtc/cgggtttaaacgactatttctttgcttgctctg; TLR2, cccttcgaaacagcactggtgtctggc/cgggtttaaacaacctaggactttatcgacg; TLR3, cccttcgaagaactctttttcatgatcaatac/cgggtttaaacaatttaatgtacagagtttttgga; TLR4, cccttcgaaaagaccatcattggtgtgtc/cgggtttaaactcttca- gatagatgttgcttc; TLR5, cccttcgaaaagtttctccctttttcattgtat/cgggtttaaacgattaggagatggttgctac; TLR6, cccttcgaaaacataactctgctgatcgtc/cgggtttaaacttaagatttcacatcattgttttc; TLR7, cccttcgaagatctgactaacctgattctg/cgggtttaaacgaagggctagaccgtttcc; TLR8, cccttcgaagatgtcactgcagtgatattat/cgggtttaaacaacgtcagttagtattgcttaat; TLR9, cccttcgaagactgtttcgccctctcg/cgggtttaaactccggctcacggctattc; TLR10, cccttcgaaaacacagctctgttgattgt/cgggtttaaacggactgtgggatttttataga.

All PCR products were each cloned into the *Sfu*I/*Pme*I site of the pαv/myc and pβ5/MH vectors, in place of the myc/His-coding region, to produce the pαvTLR and pβ5TLR expression vectors (Fig. 1A). cDNA encoding the dominant negative MyD88(152–292) was amplified by RT-PCR from the THP-1 cDNA with the following primer pair (5′-3′), i.e. gtggaattccaccatggaccccctgggcatatg/gaagggcccgggcaggacaaggcctt, and cloned into the *Eco*RI/*Apa*I site of the pcDNA3.1 vector (pMyD88DN). The pGFP-TLR4 vector was constructed as follows. A DNA fragment encoding the signal peptide and the N-terminal 5 residues of mature human β5 was amplified from the pβ5/MH plasmid using a primer pair (5′-3′), i.e. tggtggaattcccgcggagtgcagcga/tatgttgagacctgcgagcc. cDNA encoding GFP was amplified from the pEGFP-C1 plasmid (Clontech) using the following primer pair (5′-3′): gtgagcaagggcgaggag/ttttgttcgaacttgtacagctcgtccatgc. The two DNA fragments were digested with *Eco*RI and *Sfu*I, respectively, and simultaneously ligated into the *Eco*RI/*Sfu*I site of pβ5TLR4 in place of the β5 sequence. All expression vectors were verified by DNA sequencing.

2.3. Protein purification and Western blotting

293T cells were co-transfected with the pαv/myc and pβ5/MH plasmids and cultured in the VP-SFM serum-free medium (Life Technologies) for 48 h. The culture medium was then incubated with Ni-NTA-agarose (Qiagen, Hilden, Germany) overnight at 4°C and was, after washing, eluted with imidazole following the manufacturer's instructions except that $CaCl_2$ and $MgCl_2$ were included at 1 mM in all buffers. The purified proteins were separated on 7.5% (w/v) SDS–PAGE gels and, upon electroblotting onto PVDF membranes, detected with specific antibodies. The blots were developed using the ECL Western blot detection system (Amersham Pharmacia Biotech).

2.4. NF-κB luciferase assay

293T cells were sub-cultured in 24-well tissue culture plates 24 h before transfection and were co-transfected with the pαvTLR and/or the pβ5TLR plasmids using the GenePORTER 2 reagent and following the manufacturer's instructions (Gene Therapy Systems, La Jolla, CA, USA). As negative controls, the cells were transfected with the pcDNA3.1 vector. The p5×NF-κB-luc (Stratagene, La Jolla, CA) and pRL-CMV (Promega, Madison, WI, USA) luciferase reporter plasmids were co-transfected in all experiments and the quantity of plasmids has been specified in the figure legends. After 48 h, luciferase expression in the transfected cells was determined using the Dual Luciferase Assay kit (Promega).

2.5. Flow cytometry

293T cells were transfected with pαvTLR/pβ5TLR plasmid pairs to express TLR homodimers, e.g. pαvTLR1/pβ5TLR1. As controls, the cells were transfected with the pcDNA3.1 vector. The transfected cells were harvested in 36 h and, after blocking with 20% (v/v) goat serum, stained with the P1F6 antibody. Cells were washed in FACSwash (2.5% (v/v) BCS in PBS) and stained with goat anti-mouse IgG (FITC-conjugated; Sigma). Cells were also stained after permeabilization for 10 min with 0.1% saponin in FACSwash. The stained cells were analyzed on FACScalibur using the CellQuest software (Becton Dickinson Immunocytometry Systems).

2.6. Sequence analysis

Sequences of TLR1–10 cytoplasmic domains and the TIR domain of MyD88 (residues 152–292) were extracted from the following GenBank entries: U88540 (TLR1), U88878 (TLR2), U88879 (TLR3), U88880 (TLR4), AB060695 (TLR5), AB020807 (TLR6), AF245702 (TLR7), AF245703(TLR8), AF245704 (TLR9), AF196673 (TLR10), and U84408 (MyD88). Similarity between these sequences were compared using the MultAlin software (http://prodes.toulouse.inra.fr/multalin/multalin.html).

3. Results

3.1. The extracellular domains of the integrin αv and β5 subunits express as heterodimers

To examine whether TLRs are activated through dimerization, we expressed TLRs as chimeras with the extracellular domains of integrin αv and β5 which have been reported to form stable heterodimers [20]. To determine whether αvβ5 heterodimers form in our experimental system, β5 was expressed with both myc and His tags (β5-MH), whereas αv was expressed only with the myc tag (αv-myc) (Fig. 1A). Upon co-expression in 293T cells, β5-MH was purified from the culture media by affinity chromatography on Ni-NTA-agarose. Two protein bands of approximately 120 and 90 kDa were found reactive with the anti-myc antibody. Western blotting with anti-αv and anti-β5 antibodies confirmed that the larger band was αv and the smaller band was β5 (Fig. 1B). The anti-His antibody only recognized the β5 band. Therefore, despite its lack of the His tag, αv-myc was co-purified with β5-MH. Similar amounts of αv and β5 were detected with the anti-myc antibody, implying that, as expected, αv and β5 most likely formed 1:1 heterodimers.

Table 1
NF-κB activation by TLR dimers

	β5-1	β5-2	β5-3	β5-4	β5-5	β5-6	β5-7	β5-8	β5-9	β5-10
αv-1	3.10	**23.10**	2.52	**39.14**	4.79	3.90	3.14	4.55	1.97	1.17
αv-2	**22.41**	3.66	1.72	**30.97**	4.21	**28.21**	3.21	4.51	1.69	0.69
αv-3	2.45	2.21	1.90	**30.21**	2.45	2.21	2.45	2.69	1.66	0.79
αv-4	**19.45**	**19.07**	**16.35**	**31.93**	**15.83**	**18.07**	**15.07**	**14.69**	**11.28**	**13.45**
αv-5	1.97	3.00	2.07	**26.21**	3.90	2.76	2.66	2.21	1.97	1.35
αv-6	2.17	**20.97**	1.34	**20.86**	2.62	2.76	2.00	2.76	1.31	1.41
αv-7	1.79	2.31	2.41	**42.14**	2.07	1.90	3.93	4.07	1.52	1.00
αv-8	2.97	2.76	2.35	**23.72**	2.21	2.52	4.28	3.76	3.72	1.86
αv-9	1.14	1.55	1.48	**28.76**	1.97	1.76	1.90	4.41	1.04	2.31
αv-10	1.14	1.45	1.48	**31.38**	2.72	2.24	2.28	2.93	1.76	0.83
P	2.28	3.97	1.93	**21.72**	2.90	1.07	2.14	2.66	1.21	1.07

The 10 pαvTLR and 10 pβ5TLR plasmids were co-expressed in 293T cells in 100 possible pairs. The pβ5TLR plasmids were also each co-expressed with the pcDNA3.1 vector. The two luciferase reporter plasmids were co-transfected as in Fig. 2A and all plasmids were used at 250 ng/well. NF-κB activation was presented as folds of increases, taking the relative luciferase activity derived from 293T cells transfected with pcDNA3.1, in place of the TLR expression vectors, as 1. 'αv-1' denotes αv-TLR1 and 'β5-1' denotes β5-TLR1. 'P' denotes the pcDNA3.1/myc-His vector.

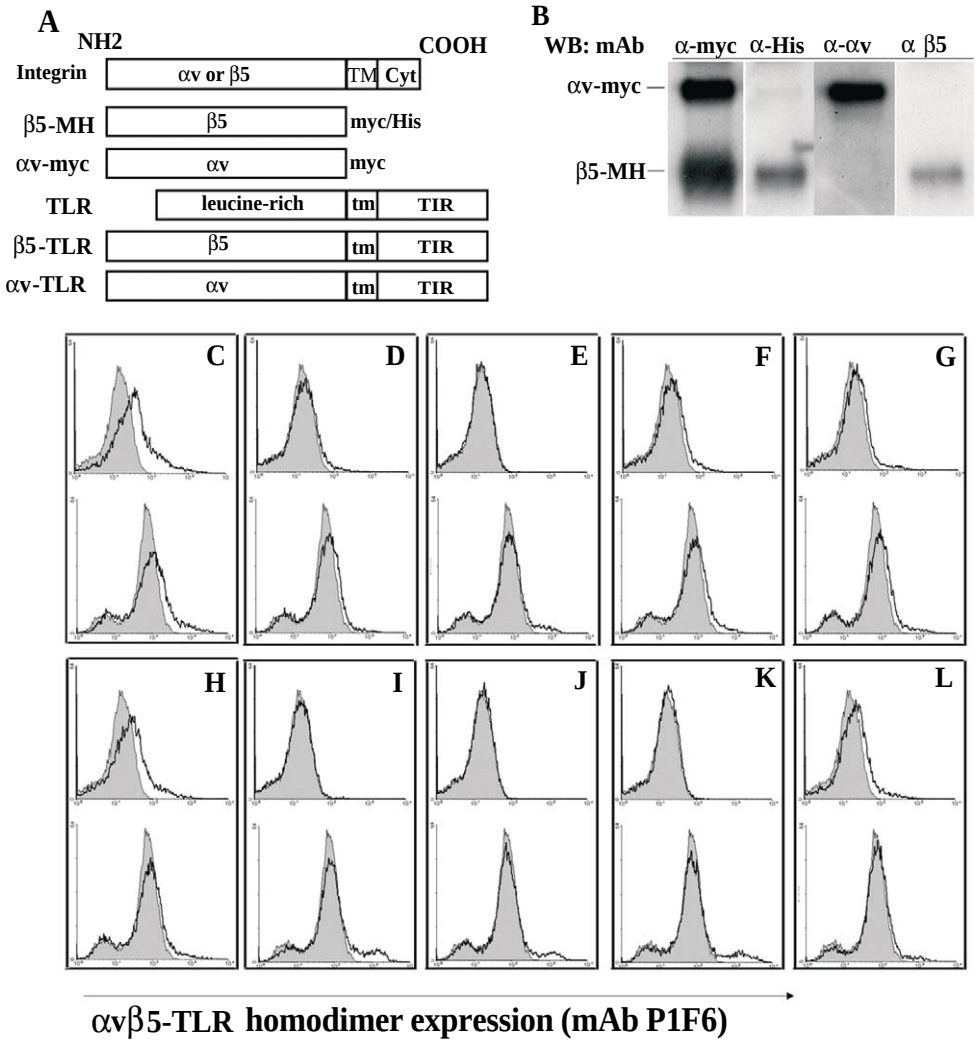


Fig. 1. Cloning and expression of the extracellular domains of integrin αv and β5 and TLR chimeras. A: cDNA encoding the αv and β5 extracellular domains of human integrin αv and β5 were amplified by PCR and cloned into the pcDNA3.1 vector as described in Section 2. β5 was cloned to express both myc and His tags at the C-termini (β5-MH), whereas a stop codon was introduced between the myc and His-coding sequences to express αv with only the myc tag (αv-myc). Sequences encoding the TLR TM/Cyt domains were cloned into the αv-myc and β5-MH expression vectors to replace the myc/His-coding sequences. B: αv-myc and β5-MH were co-expressed in 293T cells. β5-MH was isolated from the culture media using Ni-NTA-agarose and the purified proteins were detected by Western blotting (WB) using monoclonal antibodies (mAb) against the myc (α-myc) and His (α-His) epitopes and the αv (α-αv) and β5 (α-β5) integrin subunits. αv-myc and β5-MH that were detected on the blots have been indicated. C–L: Histograms obtained with 293T cells transfected to express αvβ5-mediated TLR1/1–TLR10/10 homodimers. αv-TLR and β5-TLR were co-expressed in 293T cells in pairs to produce αvβ5-mediated TLR homodimers and these dimers were detected by flow cytometry using the P1F6 mAb. Upper panels: 293T cells stained with P1F6 without membrane permeablization. Lower panels: 293T cells stained after membrane permeablization with saponin. Shaded profiles: 293T cells transfected with the pcDNA3.1 vector and stained with mAb P1F6.

3.2. *Differential sub-cellular targeting of αvβ5-TLR homodimers*

To express TLR dimers, sequences encoding the TM/Cyt domains of TLR1–10 were cloned into the pαv/myc and pβ5/MH vectors in frame with 5′ αv- and β5-coding sequences (Fig. 1A). The resultant pαvTLR and pβ5TLR vectors express αv-TLR and β5-TLR chimeras and the ability of these chimeras to form αvβ5-mediated dimers were examined. To express TLRs as αvβ5-mediated homodimers, each pαvTLR vector was co-expressed with a corresponding pβ5TLR vector in 293T cells and αvβ5 dimers was detected by flow cytometry using the P1F6 antibody. The antibody detected the expression of chimeric, αvβ5-mediated TLR1, TLR6, TLR10 and, to a lesser extent, TLR2, TLR4 and TLR5, but not TLR3, TLR7–9 homodimers on the cell surface (Fig. 1 C–L, upper panels). However, after the cells were permeablized with saponin, a distinct intracellular pool for each of TLR3 and TLR7–9 was detected (Fig. 1E, I–K, lower panels). This is suggestive of the ability of the TM/Cyt domains to target these four TLRs to intracellular compartments. It is not known whether this TM/Cyt domain-directed TLR targeting is also dependent on dimerization. Nevertheless, it was shown that all the 10 αv-TLR and the 10 β5-TLR chimeras were expressed which also formed αvβ5-nucleated homodimers.

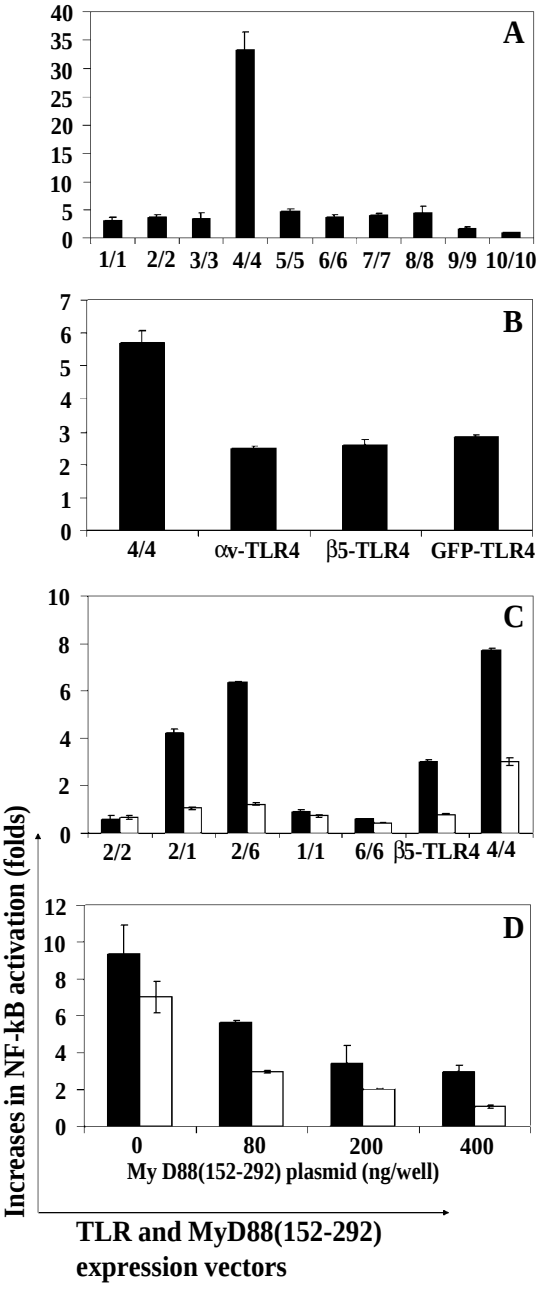
3.3. *TLR4/4 are the only TLR homodimers that can activate NF-κB*

Although all 10 TLR dimers were expressed in 293T cells,

NF-κB was only effectively activated in 293T cells that expressed the TLR4/4 homodimer (Fig. 2A). NF-κB activation by CD4-nucleated TLR4/4 homodimers has been previously reported [13,16,17]. In these earlier studies, other TLR homodimers, including TLR1/1, TLR2/2 and TLR6/6, were also found inactive. Therefore, homodimerization might not be a common or sufficient mechanism of NF-κB activation by the majority of TLRs.

3.4. TLR2/1 and TLR2/6 are the only TLR heterodimers that activate NF-κB

The lack of NF-κB activation by most TLR homodimers prompted us to examine whether TLRs would be activated through heterodimerization. As shown in Table 1, αvβ5-nucleated TLR2/1 and TLR2/6 heterodimers were able to activate NF-κB effectively, whether αv-TLR2 or β5-TLR2 was used in TLR2 heterodimer expression. TLR2 heterodimers with other TLRs were inactive (Table 1). Therefore, TLR2 activation relies on its dimerization with TLR1 or TLR6. No other TLRs could produce heterodimers that were able to activate NF-κB, implying that heterodimerization between these known TLRs might also not be sufficient to activate most of these TLRs.



3.5. TLR4 monomers and homodimers differentially activate NF-κB

As shown in Table 1, αv-TLR4 or β5-TLR4 was able to activate NF-κB in the presence of any αv-TLR. In fact, β5-TLR4 or αv-TLR4 alone was also potent in NF-κB activation (Fig. 2B). To rule out the possible experimental artifact that αv-TLR4 and β5-TLR4 dimerize with endogenous integrins which clustered the TLR4 chimeras at cell-matrix focal adhesion sites and led to TLR4 activation, the TLR4 TM/Cyt domain was expressed as a chimera with the green fluorescence protein (GFP–TLR4). As shown in Fig. 2C, GFP-TLR4 was as potent as the other TLR4 chimeras in NF-κB activation. Therefore, it is clear that TLR4 monomers can activate NF-κB. However, TLR4/4 was more potent than TLR4 monomers in NF-κB activation (Fig. 2C).

3.6. MyD88 in NF-κB activation by TLR2/1, TLR2/6, TLR4 and TLR4/4

MyD88 is a critical adaptor protein for TLR-mediated NF-κB activation [3–8]. However, LPS has also been reported to activate NF-κB through a TLR-4-dependent but MyD88-independent signaling pathway [21]. We therefore examined whether dominant negative MyD88(152–292) could inhibit NF-κB activation by these αvβ5-mediated TLR4 and TLR2 monomers/dimers. As shown in Fig. 2C, NF-κB activation by TLR2/1 and TLR2/6 was completely blocked by MyD88(152–292). NF-κB activation by the TLR4 monomers was also completely inhibited (Fig. 2C). MyD88 inhibited NF-κB activation by TLR4 monomers and TLR4/4 homodimers in a dose-dependent manner (Fig. 2D). However, residual NF-κB

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Fig. 2. NF-κB activation by TLR monomers and αvβ5-mediated TLR dimers. A: αv-TLR and β5-TLR were co-expressed in 293T cells as in Fig. 1C and NF-κB activation by these TLR monomers and homodimers was determined using a luciferase assay. '1/1' represents the co-expression of αv-TLR1 and β5-TLR1. All plasmids were used at 250 ng/well. B: 293T cells were either co-transfected with pαvTLR4 and pβ5TLR4 each at 150 ng/well (TLR4/4) or singly transfected with pαvTLR4, pβ5TLR4 or pGFP-TLR4 at 300 ng/well and NF-κB activation was similarly determined using the luciferase assay. C: NF-κB activation by TLR2 and TLR4 dimers and inhibition by dominant negative MyD88. 293T cells were transfected as in A except that each TLR plasmid was used at 150 ng/well. The cells were co-transfected with the pMyD88DN plasmid (open bars) and, as controls, the pcDNA3.1 vector (solid bars) at 400 ng/well. The p5×NF-κB-luc and pRL-CMV luciferase reporter plasmids were co-transfected in all experiments at the same dosage as the TLR expression vectors. After 48 h, the activity of NF-κB-directed firefly luciferase was determined and normalized to the CMV-directed *Renilla* luciferase activity and expressed as folds of NF-κB activities derived from 293T cells that were transfected with the pcDNA3.1 vector. All results are representative of at least three similar experiments and are presented as means ± S.D. D: 293T cells were transfected as in C except that the pMyD88DN [MyD88(152–292)] plasmid (open bars) or, as controls, the pcDNA3.1 vector (solid bars) was used at different dosages (0 ng, 80 ng, 200 ng and 400 ng).

activation by TLR4 homodimers was consistently observed in the presence of MyD88(152–292). This suggests that the TLR4 homodimer, but not the TLR4 monomer, is able to activate NF-κB through an additional, MyD88-independent signaling pathway.

4. Discussion

The use of integrin αvβ5 to nucleate TLR dimerization enabled us to examine all possible TLR dimers in the activation of NF-κB. Our results are consistent with previous reports that NF-κB can be activated by TLR2 and TLR4 dimers. No additional TLR dimers have been identified in this study that also activate NF-κB. This implies that dimerization between these known TLRs might not be sufficient to activate NF-κB, although it cannot be directly tested whether TLR dimers thus expressed adopt conformations expected from wild type TLRs. Nevertheless, this strategy of TLR dimer expression also led to the realization that TLR4 monomers could also effectively activate NF-κB. It is not clear how TLR4 monomers activate NF-κB. However, comparing TLR cytoplasmic sequences with that of the MyD88 TIR domain clearly indicated that the latter was most closely related to the TLR4 cytoplasmic domain (Fig. 3). Over-expression of MyD88 can activate NF-κB and over-expression of wild type TLR4 has also been shown to activate its signaling [22]. It is possible that the TIR domains in MyD88 and TLR4 represent a more recent duplication with both being able to activate signaling upon elevated expression.

It was also observed in this study that NF-κB activation by the TLR4 monomer was completely inhibited by MyD88(152–292). In contrast, NF-κB activation displayed by TLR4 homodimers was not completely inhibited. Two possible mechanisms are emerging that may explain this residual, MyD88-independent NF-κB activation by TLR4 homodimers. In the absence of MyD88, TLR4 may activate NF-κB through the recently identified MyD88-adaptor-like (Mal) protein [22]. However, Mal-mediated NF-κB could be inhibited by MyD88(152–292) [22], whereas the residual NF-κB activation by TLR4 homodimers observed in this study was not blocked by MyD88(152–292; Fig. 2C). The other possible mechanism that is potentially responsible for this residual, MyD88-independent NF-κB activation by TLR4 homodimers, may involve the activation of interferon-regulatory factor 3 [23].

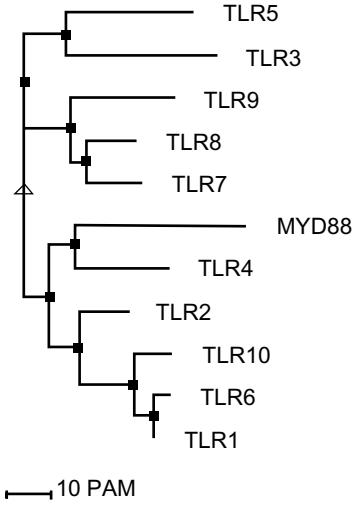

Fig. 3. Phenogram tree presentation of sequence similarity between TLR cytoplasmic domains and the MyD88 TIR domain. Sequences were extracted from the same GenBank entries based on which the αv-TLR, β5-TLR and MyD88(152–292) expression vectors were constructed.

Based on the above results, it may be proposed that TLR4 activates NF-κB following two distinct mechanisms. When TLR4 is expressed at low levels, it may only be activated by PAMP agonists through homodimerization which presumably activates NF-κB through both MyD88-dependent and -independent signaling pathways [21]. When TLR4 is persistently expressed at high levels, such as in atherosclerosis and inflammatory bowel diseases [24,25], it probably activates NF-κB in a manner independent of obvious pathogen challenges which is characteristic of chronic inflammation. Over-expression of wild type TLR4 has indeed been found to activate NF-κB in the absence of specific PAMP agonist [22]. Therefore, individuals who tend to exhibit enhanced or persistent TLR4 expression may be more susceptible to chronic inflammatory conditions.

It was clear from this study that homodimers of TLR3 and TLR7–9 were only detected intracellularly, whereas the other TLRs were detected on the cell surface, and this appears to be determined by the cytoplasmic domains of TLRs. Interestingly, comparison between cytoplasmic domains of all 10 human TLRs clearly grouped TLR7–9 as a distinct subfamily and TLR1, TLR6 and TLR10 as another (Fig. 3). It is not known whether cytoplasmic domain-mediated subcellular TLR targeting is also regulated through monomer–dimer transition. However, this set of expression constructs would allow further study of the mechanism by which subcellular targeting of TLRs is regulated.

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