Supplementary Information

Design of synthetic oligoribonucleotide-based agonists of Toll-like receptor 3 and their immune response profiles *in vitro* and *in vivo*

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Notes

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Supplementary Information

SI Table 1. Sequences, analytical data and Tms of different lengths of dsORNs

dsORN Compound Number	Sequence[a]	Length of ORN strands	Length of alignment & oligo I/C segments[b]	Purity (%)[c] HPLC	Purity (%)[c] CGE	MALDI-TOF Mass[d] Calc.	MALDI-TOF Mass[d] Found	Duplex stability Tm (^{o}C)[e]	Hyper-chromicity (%)
	5’-GCAGUUGACA$(C)_{30}$-3’	40-mer	10-30	95	91	12340	12328	57.9/48.6	27.0
	5’-UGUCAACUGC$(I)_{30}$-3’	40-mer	10-30	97	90	13027	13014		
	5’-CACUGGCAGUUGACA$(C)_{35}$-3’	50-mer	15-35	96	97	15456	15468	71.9/50.8	26.9
	5’-UGUCAACUGCCAGUG$(I)_{35}$-3’	50-mer	15-35	98	94	16309	16307		
	5’-CACUGGCAGUUGACACAGGU$(C)_{40}$-3’	60-mer	20-40	96	90	18613	18633	77.3/51.2	22.7
	5’-ACCUGUGUCAACUGCCAGUG$(I)_{40}$-3’	60-mer	20-40	95	91	19551	19562		

[a]: All ORNs are synthesized with phosphodiester backbone; [b]: first and second numbers indicate nucleotide length of alignment and oligo I/oligo C segments, respectively; [c]: purity is expressed as % full-length product with the rest being one or two nucleotides short as determined by anion-exchange HPLC and CGE (capillary gel electrophoresis); [d]: Mass was determined by MALDI-TOF mass spectral analysis, Calc. and Found indicate calculated and experimentally determined values, respectively; [e]: thermal melting stability of duplexes was determined by UV thermal denaturation curves at 1 μM concentration of duplex in 150 mM sodium chloride, 10 mM sodium hydrogen phsophate buffer, pH 7.2; data are representative of two independent experiments; the two Tm values correspond to duplex of alignment segments and duplex of oligo I/oligo C segments, respectively.

SI Table 2. Sequences, analytical data and Tms of 50-mer dsORNs with different lengths of alignment segments

dsORN Compound Number	Sequence[a]	Length of alignment &oligo I/C segments[b]	Purity (%)[c] HPLC	Purity (%)[c] CGE	MALDI-TOF Mass[d] Calc.	MALDI-TOF Mass[d] Found	Duplex stability Tm (^{o}C)[e]	Hyper-chromicity (%)
1	5'-$(C)_{50}$-3'	0-50	97	93	15197	15188	-/54.1	18.4
	5'-$(I)_{50}$-3'	0-50	96	90	16448	16461		
2	5'-CACUG$(C)_{45}$-3'	5-45	95	92	15262	15272	25.0/52.0	17.3
	5'-CAGUG$(I)_{45}$-3'	5-45	95	90	16428	16432		
3	5'-CACUGGCAGU$(C)_{40}$-3'	10-40	95	90	15367	15368	51.0/51.0	18.1
	5'-ACUGCCAGUG$(I)_{40}$-3'	10-40	98	91	16368	16358		
4	5'-CACUGGCAGUUGACA$(C)_{35}$-3'	15-35	96	97	15456	15468	71.9/50.8	26.9
	5'-UGUCAACUGCCAGUG$(I)_{35}$-3'	15-35	98	94	16309	16307		
5	5'-CACUGAGACUGAUGCCA$(C)_{33}$-3'	17-33	95	90	15480	15479	73.9/50.0	24.2
	5'-UGGCAUCAGUCUCAGUG$(I)_{33}$-3'	17-33	95	92	16300	16301		
6	5'-CACUGGCAGUUGACACAGGU$(C)_{30}$-3'	20-30	95	90	15561	15567	78.6/50.1	19.4
	5'-ACCUGUGUCAACUGCCAGUG$(I)_{30}$-3'	20-30	97	93	16249	16252		
7	5'-CACUGGCAGUUGACACAGGUUCCUCACUUC$(C)_{20}$-3'	30-20	96	93	15589	15584	86.1/44.2	24.7
	5'-GAAGUGAGGAACCUGUGUCAACUGCCAGUG$(I)_{20}$-3'	30-20	96	94	16296	16301		
8	5'-CACUGGCAGUUGACACAGGUUCCUCACUUCACAAAUCGUU$(C)_{10}$-3'	40-10	96	91	15728	15732	84.6/24.0	29.9
	5'-AACGAUUUGUGAAGUGAGGAACCUGUGUCAACUGCCAGUG$(I)_{10}$-3'	40-10	98	93	16201	15199		
9	5'-CACUGGCAGUUGACACAGGUUCCUCACUUCACAAAUCGUUCAUCG$(C)_{5}$-3'	45-5	97	92	15794	15801	86.9/<10	31.2
	5'-CGAUGAACGAUUUGUGAAGUGAGGAACCUGUGUCAACUGCCAGUG$(I)_{5}$-3'	45-5	95	90	16182	16187		
10	5'-CACUGGCAGUUGACACAGGUUCCUCACUUCACAAAUCGUUCAUCGUUCAC-3'	50-0	95	91	15820	15827	85.0/-	29.0
	5'-GUGAACGAUGAACGAUUUGUGAAGUGAGGAACCUGUGUCAACUGCCAGUG-3'	50-0	95	91	16186	16200		

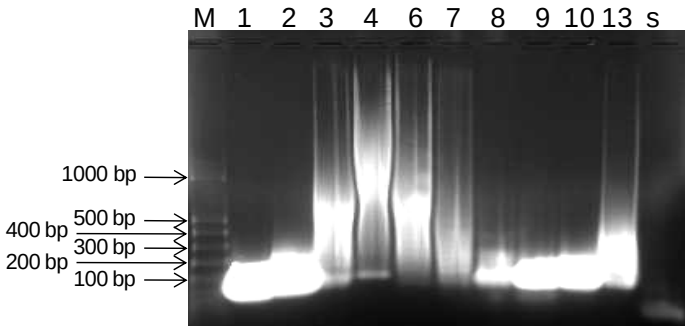
[a]: All ORNs are synthesized with phosphodiester backbone; [b]: first and second numbers indicate nucleotide length of alignment and oligo I/oligo C segments, respectively; [c]: purity is expressed as % full-length product with the rest being one or two nucleotides short as determined by anion-exchange HPLC and CGE (capillary gel electrophoresis); [d]: mass was determined by MALDI-TOF mass spectral analysis, Calc. and Found indicate calculated and experimentally determined values, respectively; [e]: thermal melting stability of duplexes was determined by UV thermal denaturation curves at 1 μM concentration of duplex in 150 mM sodium chloride, 10 mM sodium hydrogen phsophate buffer, pH 7.2; data are representative of two independent experiments; the two Tm values correspond to duplex of alignment segments and duplex of oligo I/oligo C segments, respectively.

SI Table 3. Sequences, analytical data and Tms of 50-mer dsORNs with different sequence compositions of alignment segments and chemical modifications in oligo I/oligo C segments

dsORN Compound Number	Sequence[a]	Length of alignment & oligo I/C segments[b]	Purity (%)[c] HPLC	Purity (%)[c] CGE	MALD-TOF Mass[d] Calc.	MALD-TOF Mass[d] Found	Duplex stability Tm (^{o}C)[e]	Hyper-chromicity (%)
11	5'-CACUGAGACUGAUGC$(C)_{35}$-3'	15-35	96	92	15456	15457	69.0/52.0	26.1
	5'-GCAUCAGUCUCAGUG$(I)_{35}$-3'	15-35	96	93	16309	16304		
12	5'-CAAGGCAAGCAUUCG$(C)_{35}$-3'	15-35	95	91	15479	15477	69.0/53.0	26.1
	5'-CGAAUGCUUGCCUUG$(I)_{35}$-3'	15-35	95	95	16286	16278		
13	5'-CAAUGGCACUUAACA$(C)_{35}$-3'	15-35	94	93	15448	15430	32.0/52.0	21.0
	5'-UGUCAACUGCCAGUG$(I)_{35}$-3'	15-35	98	97	16309	16307		
14	5'-CACUGGCAGUUGACA$(C)_{35}$	15-35	96	97	15456	15468	71.0/56.3	19.0
	5'-UGUCAACUGCCAGUG$(I)_{10}$**G***$(I)_{9}$**G***$(I)_{9}$**G***$(I)_{4}$	15-35	98	93	16351	16362		
15	5'-CACUGGCAGUUGACA$(C)_{4}$**C***$(C)_{9}$**C***$(C)_{9}$**C***$(C)_{10}$	15-35	93	90	15498	15488	70.2/51.1	21.8
	5'-UGUCAACUGCCAGUG$(I)_{35}$	15-35	98	94	16307	16307		

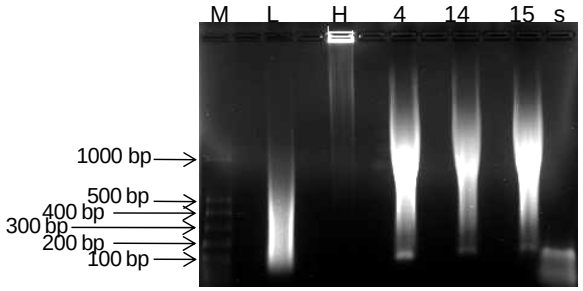
[a]: All ORNs are synthesized with phosphodiester backbone ; Underlined nucleotides indicate mismatches, **G*** and **C*** indicate 7-deazaguanosine and 5-methylcytidine, respectively; [b]: first and second numbers indicate nucleotide length of alignment and oligo I/oligo C segments, respectively; [c]: purity is expressed as % full-length product with the rest being one or two nucleotides short as determined by anion-exchange HPLC and CGE (capillary gel electrophoresis); [d]: mass was determined by MALDI-ToF mass spectral analysis, Calc. and Found indicate calculated and experimentally determined values, respectively; [e]: thermal melting stability of duplexes was determined by UV thermal denaturation curves at 1 μM concentration of duplex in 150 mM sodium chloride, 10 mM sodium hydrogen phsophate buffer, pH 7.2; data are representative of two independent experiments; the two Tm values correspond to duplex of alignment segments and duplex of oligo I/oligo C segments, respectively.

SI Figure 1



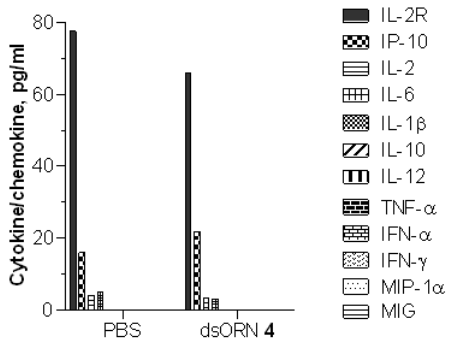
SI Figure 1. Agarose gel electrophoresis of dsORNs **1**-**4**, **6**-**10** and **13**. Please see Table 1 for dsORN sequences. M stands for double-stranded RNA length markers. Numbers on the top of lanes correspond to dsORN numbers shown in Table 1. s Stands for oligo C strand of dsORN **4**.
Agarose gel (1%) containing 0.05% (v/v) ethidium bromide was loaded with about 1 OD of dsORNs and carried out electrophoresis using 1 X Tris borate EDTA buffer, pH 9.0 at a constant voltage of 90v for 3.5 hours in cold room. After the electrophoresis gel was placed on UV transilluminator and a photograph was taken.

SI Figure 2



SI Figure 2. Agarose gel electrophoresis of dsORNs **4**, **14** and **15**. Please see Table 1 for dsORN sequences. M stands for double-stranded RNA length markers. Numbers on the top of lanes correspond to dsORN numbers in Table 1. L, H, and s stand for low molecular weight poly I:C (Invivogen), high molecular weight poly I:C (Invivogen) and oligo C strand of dsORN **4**.
Agarose gel (1%) containing 0.05% (v/v) ethidium bromide was loaded with about 1 OD of dsORNs, low and high molecular weight poly I:C and carried out electrophoresis using 1 X Tris borate EDTA buffer, pH 9.0 at a constant voltage of 90v for 3.5 hours in cold room. After the electrophoresis gel was placed on UV transilluminator and a photograph was taken.

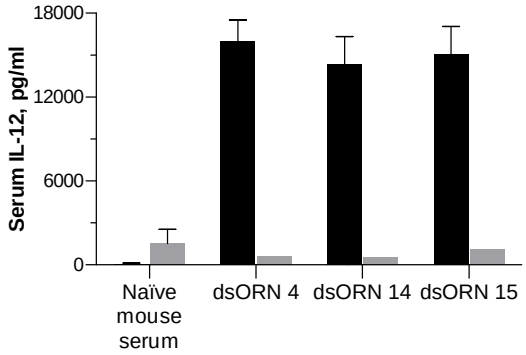
SI Figure 3



SI Figure 3. dsORN **4** does not induce cytokine responses in human pDCs. Human pDCs were cultured in the presence or absence of dsORN **4** at 500 μg/ml concentration for 24 hr. Cell culture supernatants were analyzed by multiplex assay. PBS was used as control. Data shown are representative of two independent experiments.

Human pDCs (10^6/ml) were plated into 96-well plates. dsORN **4** dissolved in PBS was added at 500 μg/ml concentration. The cells were then incubated at 37°C for 24 hours. The levels of cytokines and chemokines in the culture supernatants were measured using a human multiplex kit on the Applied Cytometry Systems Luminex 100/200 instruments, and the data were analyzed using StarStation software, version 2.0. The required reagents were purchased from Invitrogen (Carlsbad, CA).

SI Figure 4



SI Figure 4. Serum IL-12 induction by dsORNs **4**, **14** and **15** in wild-type (black bars) and TLR3$^{-/-}$ (grey bars) C57BL/6 mice. dsORNs were administered subcutaneously at a dose of 10 mg/kg. Blood was collected 2 hr post dsORN administration and serum IL-12 levels were measured by ELISA. Naïve wild-type and knock-out mice serum was used as control. Data shown are mean of three mice ± SD and are representative of two independent experiments.