Supplementary Note

Trans-splicing-mediated Correction of Mutant CD40L mRNA *In Vitro*

Initial experiments were performed with *trans*-splicer targeted against the 3' end of CD40L intron 1 (hybridization domain "d" in **Fig. 1**). The term "hybridization domain" refers to a sequence complementary to the intron target in CD40L pre-mRNA which will allow the *trans*-splicer and target pre-mRNA to "hybridize" to facilitate splicing (**Supplementary Fig. 1a** online). RT-PCR performed on RNA from cells transfected with both target plasmids and *trans*-splicer using a primer set specific for *trans*-spliced RNA indicated that *trans*-splicing had occurred (**Supplementary Fig. 1b**, lane 1 online). In contrast, using a primer set specific for *trans*-spliced CD40L, no RT-PCR product was seen from cells transfected with target plasmid alone (lane 2), *trans*-splicer plasmid alone (lane 3), target plus *trans*-splicer plasmids without reverse transcription (lane 4), pNull (identical to the *trans*-splicer plasmid, but containing no transgene; lane 5), and target plus a *trans*-splicer plasmid lacking the hybridization domain (lane 6), suggesting that RNA *trans*-splicing and not DNA recombination was responsible for the observed correction of CD40L mRNA.

Preliminary data indicated that co-transfection of *trans*-splicer plasmids containing a hybridization domain 312 nt in length plus the target plasmid resulted in the highest efficiency of *trans*-splicing (not shown). To further improve the efficiency of *trans*-splicing, several *trans*-splicer plasmids were created with hybridization domains targeted to different locations within CD40L intron 1 (labeled a-d, **Fig. 1d**) using the optimal size of 312 nt in each case. Co-transfection of *trans*-splicer containing hybridization domains a, b, c or d and target plasmid resulted in 3.2, 10.4, 3.0 and 1.7% of total CD40L splicing respectively, as determined by real time quantitative RT-PCR (mean, **Supplementary Fig. 2** online). Thus, hybridization domain b, located 432 nt from the 5' end of CD40L intron 1, was the most efficient hybridization domain tested. To assess the fidelity of CD40L *trans*-splicing, the RT-PCR products from

these reactions were sequenced. CD40L mRNA corrected by *trans*-splicing *in vitro* contained the correct sequence comprised of normal exons 4 and 5, and contained the expected sequence at the splice junction between target and *trans*-splicer transcripts (not shown), demonstrating that *trans*-splicing occurred correctly *in vitro*.

CD40L Protein Expression from *Trans*-splicing *In Vitro*

To assess whether CD40L corrected by *trans*-splicing in HeLa cells resulted in expression of the protein on the cell surface, flow cytometry was performed on cells transfected with pCD40L (positive control), pNull plus target plasmid (negative control) or *trans*-splicer containing hybridization domain b and target plasmids. The data revealed that 49.0, 1.0 and 14.9% of the cells were CD40L-positive (mean, **Supplementary Fig. 3a** online) and mean fluorescent intensities (MFI) were 22.3, 2.6 and 4.4, respectively (not shown). CD40L *trans*-splicing as a percent of control was 9.3% in HeLa cells transfected with *trans*-splicer and target plasmids (not shown). These results are consistent with the real-time RT-PCR data, demonstrating that CD40L was expressed on the surface of transfected HeLa cells.

Trans*-spliced CD40L Functions Normally *In Vitro

CD40L is involved in B cell proliferation and required for IgE production[1,2]. The proliferation of B cells was studied *in vitro* as a function of CD40L expression using carboxy-fluorescein diacetate, succinimidyl ester (CFSE) to evaluate cell division, in which dye staining is reduced in dividing cells[3]. To evaluate the effect of *tran*s-spliced CD40L on B cell division, 36 hr after transfection with *trans*-splicer and target plasmids, HeLa cells were irradiated and co-cultured with CFSE-labeled murine splenic B cells. From 1 to 7 days after initial co-culture, mixed cells were examined by flow cytometry with B cells (B220-positive cells) and dividing cells identified as low CFSE-staining cells. After 7 days, 87.9% of B cells co-cultured with HeLa cells transfected with pCD40L plasmids had proliferated (**Supplementary Fig. 3b** online). In contrast, only 7.3% of B cells co-cultured with HeLa cells

transfected with pNull had proliferated. Importantly, 24.2% of B cells co-cultured with HeLa cells transfected with *trans*-splicer and target plasmids had proliferated (**Supplementary Figure 3b** online), demonstrating that *trans*-spliced CD40L stimulated proliferation of B cells. The effect on B cell proliferation was evident from 4 days after co-culture (not shown).

To evaluate the effect of *trans*-spliced CD40L on production of IgE from B cells, B cells were co-cultured for 6 days with irradiated HeLa cells transfected with either pNull, *trans*-splicer plus target plasmids, or pCD40L plasmid. Concentrations of IgE in the conditioned media were examined using ELISA. B cells alone or B cells plus HeLa cells transfected with pNull showed low IgE concentrations (0.7 ng/ml and 1.9 ng/ml, mean, respectively, **Supplementary Fig. 3c** online). In contrast, B cells in contact with HeLa cells transfected with *trans*-splicer and target plasmids demonstrated increased IgE production (mean, 46.5 ng/ml, **Supplementary Fig. 3c** online), indicating that *trans*-spliced CD40L had restored normal B cell function *in vitro*.

To assess the effect of *trans*-spliced CD40L on the activation of macrophages, RAW 264.7 murine macrophage-like cells were co-cultured for 6, 24 or 48 hr with irradiated HeLa cells transfected with either pNull, *trans*-splicer plus target plasmids, or pCD40L plasmid. Concentrations of TNF-_ in the conditioned medium were determined using ELISA and used as an index of macrophage activation. After 24 and 48 hr, RAW 264.7 cells co-cultured with HeLa cells transfected with *trans*-splicer plus target plasmids demonstrated increased TNF-_ production relative to pNull ($p < 0.005$ at 24 hr and $p < 0.01$ at 48 hr, target + *trans*-splicer *vs* Target + pNull, **Supplementary Fig. 3d** online) demonstrating that *trans*-spliced CD40L activated macrophages in a similar manner to normal CD40L.

To assess the possibility that peptides could be produced from accidental open reading frames within the *trans-s*plicer mRNA, we performed *in vitro* translation with *trans*-splicer pre-mRNA using ^{35}S methionine. In contrast with CD40L mRNA, *trans*-splicer mRNA

produced no detectable products, and ^{35}S incorporation into TCA-precipitable peptides was indistinguishable from background levels, suggesting negligible peptide production from *trans*-splicer pre-mRNA (not shown).

Supplemental Note References

1. Armitage, R. J. *et al.* Molecular and biological characterization of a murine ligand for CD40. *Nature* **1992 May 7;357**, 80-82 (1992).
2. Renshaw, B. R. *et al.* Humoral immune responses in CD40 ligand-deficient mice. *J Exp Med* **180**, 1889-1900 (1994).
3. Hasbold, J., Lyons, A. B., Kehry, M. R. & Hodgkin, P. D. Cell division number regulates IgG1 and IgE switching of B cells following stimulation by CD40 ligand and IL-4. *Eur. J Immunol.* **28**, 1040-1051 (1998).