Supporting Information

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SI Methods

Gene Array Experiments. Human CD4 T cells were isolated as described, and incubated in 24-well plates (Nunc), 4×10^6 cells/mL with plate-bound anti-human CD3 (OKT3) at 2 μg/mL. The stimulation was performed in RPMI medium supplemented with 0.1% BSA. After 2 h of stimulation with or without cefuroxime (50 μg/mL) or ampicillin (50 μg/mL), cells were collected washed and suspended in TRI Reagent (Molecular Research Center). RNA was extracted from samples and used to prepare probes for gene array in accord with the manufacturer's instructions (SuperArray Bioscience). Adequate labeling of the probes was tested before hybridization. Three healthy donors were tested in stimulation with cefuroxime. The membranes were analyzed online with the Image Data Acquisition and Expression Analysis (SuperArray Bioscience).

Real-Time PCR Analysis. To verify the results of the gene array, we synthesized real-time PCR primers (designed with the LightCycler probe design software; Roche). Real-time PCR of six selected genes was performed using a LightCycler (Roche). RNA was reverse-transcribed to cDNA from 1 μg of total RNA, which was then subjected to quantitative RT-PCR performed essentially according to the manufacturer's instructions. Specific primer pairs were used to amplify specific genes in the presence of 3 mM $MgCl_2$. PCR was performed in triplicate in a total volume of 20 μL of LightCycler HotStart DNA SYBR Green I mix (Roche) containing primer and 5 μL of cDNA. PCR amplification was preceded by incubation of the mixture for 10 min at 95 °C, and the amplification step consisted of 45 cycles of denaturation, annealing, and extension. Denaturation was performed for 15 s at 95 °C; annealing was performed in 60 °C; and the extension was performed at 72 °C for 20 s, with fluorescence detection at 72 °C after each cycle. After the final cycle, melting-point analyses of all samples were performed within the range of 62–95 °C with continuous fluorescence detection. A standard curve was generated from one sample in each run. Expression levels of β2-microglobulin (B2M) were used for sample normalization (β-actin levels were affected by cefuroxime treatment). The primer sequences were B2M sense TAGCTCTAGGAGGGCTG antisense ACCACAACCATGCCTTA; ACVR2 sense ATCTCCGCGTAAGGAA, antisense TGGGACTAACAATCGTG; CCR4 sense TCCTAGAGACCCTGGTG, antisense GGACTGCGTGTAAGATG; JAK1 sense AGGAGTATTACACCGTCAAG, antisense GGGTTGGGCCTATCAT; STAT4 sense ACATCCTGCGAGACTAC, antisense CACCGCATACACACTT; TLR2 sense CTTCTGGAGCCCATTG, antisense ACGGTACATCCACGTAG; NFKBIE sense GACTTTGTGGTAGAGGCA, antisense AAAACGTGGAGTCAGC. Results for each gene are presented as the relative expression level compared with B2M. Comparison between membranes was performed after normalization in accord with the manufacturer instructions.

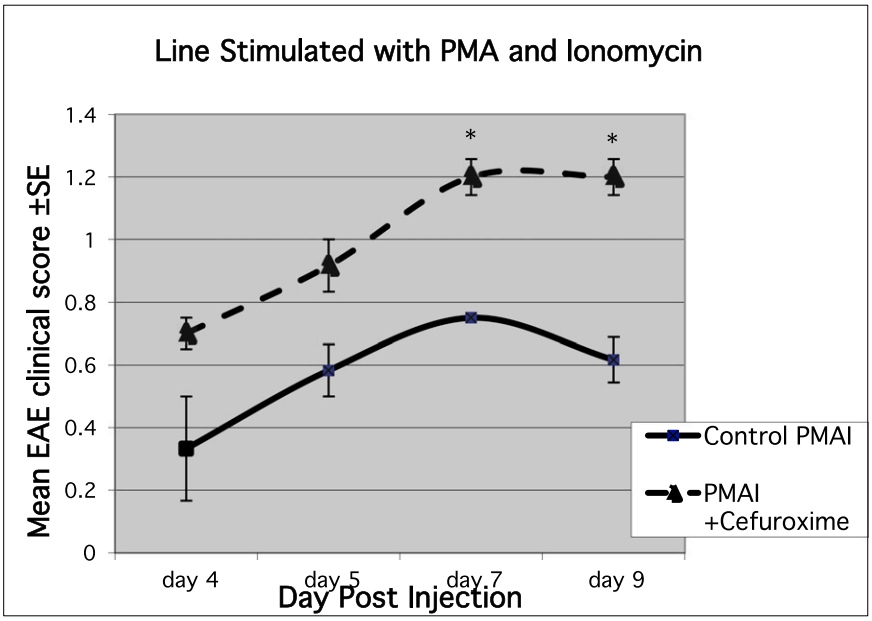

Fig. S1. Cefuroxime enhances line-induced experimental autoimmune encephalomyelitis (EAE) in the absence of antigen-presenting cells. Encephalitogenic T-cell line was stimulated for 3 d with phorbol myristate acetate (50 ng/mL) and ionomycin (500 ng/mL) with or without cefuroxime (50 μg/mL). Following stimulation, 10^7 cells were injected to Lewis rats, and EAE signs were recorded. Asterisks indicate significant ($P < 0.05$) changes between cefuroxime and control groups.

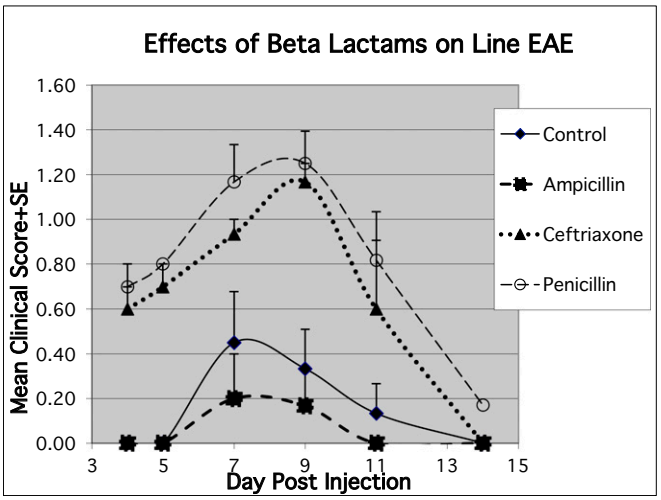

Fig. S2. Effects of beta lactams on BP10 line induced EAE in Lewis rats. Groups of rats, four each, were injected i.p. with 10^7 BP10 line cells stimulated in the presence of indicated antibiotics (50 μg/mL). Disease scores were determined. Ceftriaxone and penicillin enhanced disease scores, whereas ampicillin did not augment EAE.

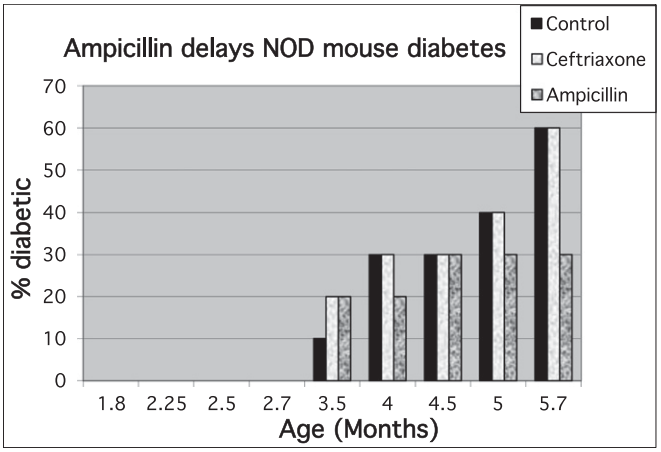

Fig. S3. Inhibition of type I diabetes in nonobese diabetic mice by ampicillin treatment. Mice were injected with ceftriaxone 675 μg or ampicillin 1,350 μg weekly, and were followed for the development of diabetes, marked by blood sugar above 300 mg/dL on two measurements.

Table S1. Clinical uses of beta-lactams studied

Beta Lactam	Family	Spectrum
Benzylpenicillin	Penicillin	*Streptococcus*
Ampicillin	Penicillin	*Streptococcus*, plus *Listeria monocytogenes*, *Enterococcus* species, *Proteus mirabilis*, and some strains of *Escherichia coli*
Cefuroxime	Cephalosporin second generation	*Moraxella catarrhalis*, *E. coli*, *P. mirabilis*, and *Klebsiella* species
Ceftriaxone	Cephalosporin third generation	Expanded coverage of Gram-negative organisms; enhanced coverage of *Proteus vulgaris* and *Providencia* species

Table S2. Effects of cefuroxime and ampicillin on gene expression by CD4+ human T cells

Gene	Full name	% decrease by cefuroxime*	% increase by ampicillin	Function†	Th1/Th2‡
Chemokines, cytokines, and their receptors					
CCR4	Chemokine receptor 4	39 ± 2	+31	Chemokine receptor	Expressed on Th2 cells (1) and on diabetogenic T cells (2)
CCR6	C-C chemokine receptor 6	53 ± 5.6	+83	-"-	Expressed on T-regulatory (3)
CCR7	Chemokine receptor 7	38 ± 8.1	+43	Bind CCL21	Expressed in EAE (4)
CCL5	Chemokine	21 ± 5.6	+25	Monocyte memory T eosinophils	Expressed in EAE lesion (5)
CXCL10	Chemokine	39 ± 3	+25	CXCR3 the receptor Attracts Th1 cells	Anti-exacerbation EAE (6) Anti protects EAE (7) and DM (8)
LTA	Lymphotoxin alpha	17 ± 10	+48.5	Cytokine	Blocking exacerbates arthritis, Th1 (9)
TNF		29 ± 8.5	+39	Cytokine	Th1, K/O mice severe EAE (10)
CCL11	Eotaxin, binds CCR3	18 ± 6.6	+107	Cytokine	Th2 attracts eosinophils (11)
SDF2	Stromal cell-derived factor	28 ± 12	+71	Secreted	??
IL-16	Lymphocyte chemoattractant factor	50 ± 7.8	+14A low expression		Treg (12)
IL-1B	Cytokine	54 ± 7.0	+64A low expression		Proinflammatory (13).
IL-9R	Receptor	27.5 ± 3	No change	Receptor	Th9 (14)
TNFRSF11A	Tumor necrosis factor receptor superfamily, activator of NF-κB	43 ± 2	+81A low expression	Membrane	??
IL-2RB	Surface	50 ± 9.9	+93	Binds IL-2	
IL-2RG	Receptor	57 ± 9.8	+78A low expression		
Surface receptors					
TLR2	Receptor	52 ± 14	+419 A low expression		EAE Exp in Treg (15)
CD28	Surface	51 ± 2.1	+175	Bind B7-1	Treg (16)
SELL	Selectin L	46 ± 7.0	+124	Adhesion to high endothelial venules	?Th1 (17)
TGF-β related					
ACVR2	Activin receptor II	60 ± 14.8	+85A low expression	Activin is TGF-β–like	
ACVR1	Activin receptor 1	42 ± 0.7	+53A low expression	Activin is TGF-β–like	
TGIF		43 ± 3.5	+72A low expression	Trans factor repress SMAD2, 3	Anti-Th2
TGFBR3	Receptor	44 ± 3.5	+139		Regulatory
SMAD7	Inhibits TGF-β	47 ± 4.2	+34A low expression		
SMAD4	TGF-β signal transduction path	47 ± 7.0	+85		
Kinases, signal transduction					
MAP3K2	Kinase	36 ± 12	+93	Reg JNK ERK5	??
MAP3K7	Kinase	46 ± 9.9	+124	TGF-β signaling NF-κB activation p38 MAPK	??
MAP3K1	Kinase	53 ± 12	+63A low expression	Activates ERK JNK	??
MAP2K4	Kinase	52 ± 14.1	+150		?Th1 (18)
MAPK9	Kinase JNK2	42 ± 10.9	+46		Th1 (19) No effect on EAE (20)
PAK1	Kinase	40 ± 2.4	+111	JNK apoptosis	??
IRAK1	Kinase	44 ± 2.1	+171		Th1 (21) IL-10 (22)
JAK1	Janus kinase 1	53 ± 13.4	+161	INF-α, -β, -γ transduction	Th1 (23) and IL-4
Transcription factors					
NFKB1		43 ± 3.5	+31	Transfer factor	Inhibit NF-κB Th2 (24)
NFKB2	Transcription factor	53 ± 10.6	+195	Lymphoma	??
NFKBIL1	Transcription factor	54 ± 12.5	+191	??	??

Table S2. Cont.

Gene	Full name	% decrease by cefuroxime*	% increase by ampicillin	Function†	Th1/Th2‡
NFKBIE	Inhibits NF-κB	63 ± 11	+132		
SRF	Serum response factor	38 ± 1.9	+58	Transfer factor	??
EGR3	Early growth response3	38 ± 2.8	−25	Transfer factor	Mitogenic activation induced in T cells, FAS-L exp (25)
JUN		49 ± 2.2	+65	Interacts with c-Fos to form a dimer	Th2 (26)
RFXAP	Regulatory factor X-associated protein	27 ± 12	+131	MHCII expression	??
CREB1	cAMP-responsive element binding protein 1	19 ± 4.9	+101	Transfer factor	??
YY1	Yin Yang 1	23 ± 9.1	+152	Transfer factor	Th2 (27) activates IL-4
REL	Transcription factor	45 ± 1.9	+132		Th1 IL-12 (28)
TRAF6	TNF receptor-associated factor 6	43 ± 3.5	+39A low expression	NF-κB and JNK activate	Limit Th2 (29)
TRAF5		43 ± 2.2	+47A low expression		Limit Th2 (30)
STAT1		32 ± 11	+85		Reg T Th1 (31)
CREBBP	Acetylates nuclear protein	40 ± 9.9	+49		
RFX5	MHCII express	36 ± 4.2	+137		
STAT4	Transcription factor	47 ± 9.3	+11		Th1 (32) Th2
SP3	Transcription factor	39 ± 9.9	+47	IL-10 control	
STAT6	Transcription factor	47 ± 7.9	No change		Th2 IL4 (33)
GFI1	Growth factor independent 1	45 ± 14	+10	Transfer factor	Th2 (34)
Others					
CD40LG	CD40 ligand	33 ± 7.1	+67	Surface B-cell interaction	Required for EAU (35) Diabetes (36)
RANBP5	Importin beta3	33 ± 3.5	+108	Nuclear protein transport	??
ACTB	Actin beta	49 ± 11	+196		
HRAS	Oncogene	30 ± 9.8	−12A low expression		??
PIN1	Isomerase	45 ± 12.1	+105		??

*Percent decrease ± SD in cefuroxime-treated human CD4 T cells relative to control.
†Cellular function of gene as found in databases.
‡Based on articles linking suggested gene to Th1, Th2, or Treg pathways; data on some genes supported evidence linking the gene to more than one pathway (e.g., CCR4, CXCL10). ?? indicates unknown function in Th1/2 polarization.

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