Development of anti-CXCL13 Monoclonal Antibody for the Treatment of Multiple Sclerosis

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Abstract

The chemokine CXCL13 is expressed in secondary lymphoid organs by follicular dendritic cells, macrophages and TH17 cells. It is the only known ligand for the CXCR5 receptor which is expressed on mature B cells, follicular helper T cells, TH17 cells and Treg cells.

Aberrant expression of CXCL13 within ectopic germinal centers has been linked to the development of autoimmune disorders (e.g. Multiple Sclerosis (MS)). In patients with secondary progressive MS, CXCL13-producing cells have been shown to accumulate in cerebral meninges, and elevated levels of CXCL13 have been found in serum and the cerebrospinal fluid of patients with Relapsing Remitting, Primary Progressive, and Secondary Progressive MS.

We hypothesize that antibody-mediated blockade of CXCL13 interaction with its receptor would interfere with formation of ectopic lymphoid follicles in the CNS and inhibit MS progression.

We developed a human IgG1 monoclonal antibody that specifically binds to human, rodent and primate CXCL13 with an affinity of approximately 5 nM. It is capable of neutralizing CXCL13 function from these various species in several *in vitro* functional assays. For *in vivo* studies we have engineered a chimeric antibody to contain the same human heavy and light chain variable regions along with mouse constant regions. This anti-CXCL13 antibody has demonstrated efficacy in ameliorating Experimental Autoimmune Encephalomyelitis (EAE) induced by either active immunization or Th17-mediated adoptive transfer. In addition, treatment with this antibody reduced the number of ectopic follicles in several different autoimmune disease models, and, in adoptive transfer studies, interfered with the trafficking of B cells to the B cell areas of spleen and lymph nodes.

The human antibody is currently undergoing pre-clinical development in preparation for filing an FDA Investigational New Drug application for potential therapy of inflammatory disease.

Generation of Anti-Human CXCL13 MAb

Swiss Webster mice immunized with KLH-conjugated human CXCL13

Hybridoma generation; selection of clone that neutralizes both, human and mouse CXCL13

3D2 (Mouse IgG1)

V genes cloning; chimeric MAb generation; conversion to fully human Ab and selection of higher affinity variants using Vaccinex ActivMAb technology. Antibody screening in specificity and functional assays

VX5/5261 (Human IgG1)

Generation of murine counterpart for *in vivo* studies: same heavy and light chain V regions as VX5/5261; murine IgG2a and kappa constant regions.

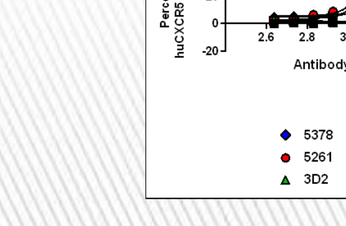
VX5/5378 (Mouse IgG2a)

Antibody Specificity for CXCL13

- Antibody affinity was determined by Biacore (on recombinant chemokines)

Antibody	Affinity, nM		
	Human CXCL13	Mouse CXCL13	Cyno CXCL13
5261	5.1	8.1	4.7
5378	4.5	4.2	NT
3D2	13	159	NT

- Specificity ELISA with recombinant antigens
  - No binding to a panel of control antigens (BSA, HAS, Insulin, hemoglobin, etc)
  - No binding to a panel of other CXC chemokines: CXCL12, CXCL8, CXCL10, and CXCL9.
- Capture Epitope Competition ELISA with native human and mouse CXCL13
  - Comparable binding to recombinant protein was detected
- Flow cytometry:
  - no non-specific binding on a panel of cell lines
- IHC on a panel of 31 normal human tissues (Non-GLP):
  - no off-target binding detected



MAb 5261-FITC binding to CXCL13 in human tonsil (A); and cynomolgus monkey spleen (B). Formalin-fixed, paraffin-embedded sections were incubated overnight with 30 ug/ml of MAb 5261-FITC or Human IgG-FITC Isotype

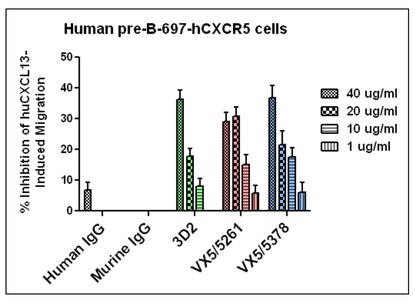
Anti-CXCL13 Antibodies Inhibit huCXCL13-Mediated Internalization of huCXCR5 Receptor

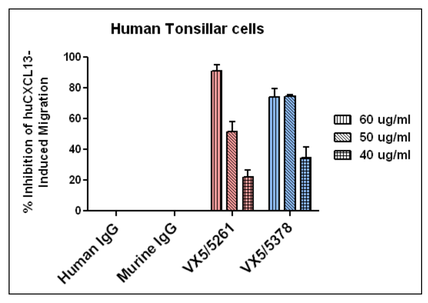
A.

B.

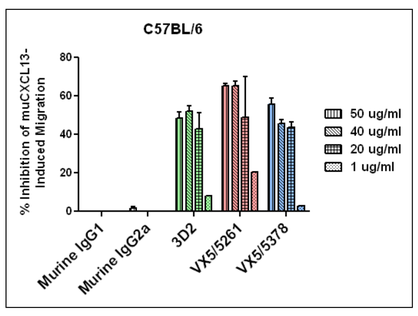
Receptor internalization assay was conducted in 96-well plate. Multiple dilutions of the antibodies (100 - 4 ug/ml) were combined with 20 ug/ml of human CXCL13; incubated O/N at 4ºC. Next day, pre-blocked (with 10 ug/ml anti-human Fc; 15 min/37ºC) human pre-B-697-hCXCR5 cells were incubated with huCXCL13/antibody mix for 2 hours at 37ºC; stained with anti-human CXCR5-APC antibody (30 minutes at 4ºC) and analyzed by flow (A). Inhibition of endocytosis (B) is calculated as follows: % Inhibition = 100 - [100*(0 CXCL13 - geomean)/(0 CXCL13 - 0 mAB)]. In graph (B) data points for 5261 and 5378 represent average of measurements from two independent experiments. Data points for 3D2 and Isotype Controls represent average of triplicate measurements from a single experiment. Curves were fitted using four-parameter sigmoidal curve fit ($R^2 = 0.99$).

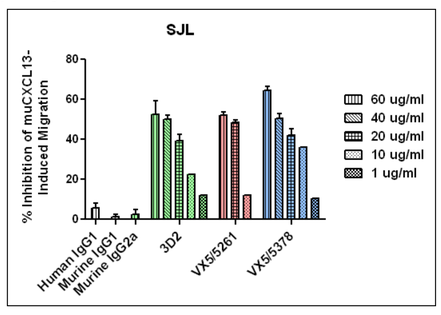
Anti-CXCL13 Antibodies Inhibit migration of Human pre-B and Tonsillar cells and Murine Splenocytes





Murine Splenocytes





$$\text{\% Migration index} = \frac{\text{(Specific migration (Chemokine + MAb or Isotype Control)}}{\text{Spontaneous Migration (No chemokine/No antibody)}}$$

$$\text{\% Migration inhibition} = \frac{\text{(Migration Index (Chemokine) – Migration Index (Chemokine + MAb))*100}}{\text{Migration Index (Chemokine)}}$$

Human Pre-B-697-huCXCR5 cells

8-um transwell plates; 5X10^6 cells/upper chamber

1 ug/ml (100 nM) huCXCL13/lower chamber

Migration time: Overnight/37ºC

Data presented as mean of seven independent experiments +/- SEM
vvvv

Human Tonsillar Cells and Murine Splenocytes

5-um transwell plates; 10^6 cells/upper chamber

5 ug/ml (500 nM) human or murine CXCL13/lower chamber

Migration time: 2 hours/37ºC

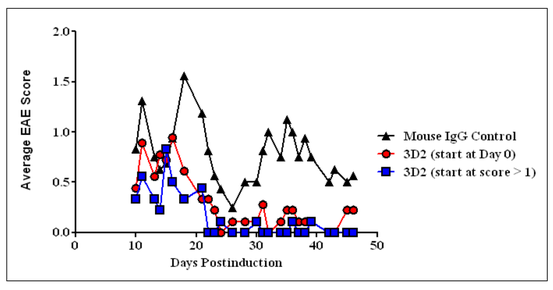
Data presented as mean of two (tonsillar cells and C57BL/6 mouse splenocytes) and eight (SJL splenocytes) independent experiments +/- SEM

None of the CXCL13 antibodies affected migration towards human or murine SDF-1α /CXCL12 (negative control; data not shown)

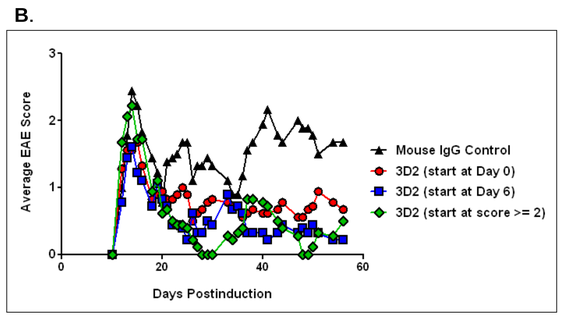
3D2 Reduces Severity of Actively-Induced Relapsing-Remitting EAE in SJL Mice

- **Relapsing-remitting (RR) disease was induced in SJL/J mice by subcutaneous immunization with PLP139-151 in 1mg/ml Complete Freund's Adjuvant (CFA) enhanced with 5mg of heat-inactivated *Mycobacterium tuberculosis* strain H37RA.**
- **Treatment consisted of bi-weekly intraperitoneal injections of 0.3 mg (15 mg/kg) of mouse anti-human CXCL13 antibody (3D2) or irrelevant Mouse IgG control**

A.



B.

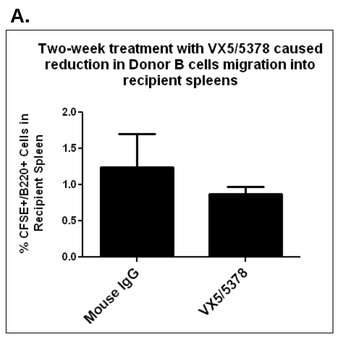


For each experiment: each data point represents a mean of scores taken from 9 mice. Group means were compared by using one-way ANOVA followed by Bonferroni's multiple comparison post test. Statistically significant differences were observed between Mouse IgG and each 3D2 treated group (P<0.05), but not among three 3D2 treated groups (P>0.05).

VX5/5378 Treatment Affects Migration of Adoptively Transferred B cells and Host Follicular Structure in Naïve BALB/c mice and Reduces Germinal Center Formation in Immunized BALB/c mice

- **Naïve BALB/c Mice:**
  - **B cells were isolated from VX5/5378 or Isotype Control treated (0.6 mg/twice a week/i.p for 2 weeks) female BALB/c mice**
  - **Donor B cells were labeled with 1 uM CFSE and injected (2X10^7 cells/i.v.) into syngeneic recipients (3 mice/group)**
  - **After 2 weeks of treatment with 0.6 mg/twice a week/i.p. of antibodies, recipients were sacrificed; spleens collected and analyzed by flow cytometry ((A) and (B); * P=0.05; ** P<0.05) or IHC on frozen section (C)**

A.



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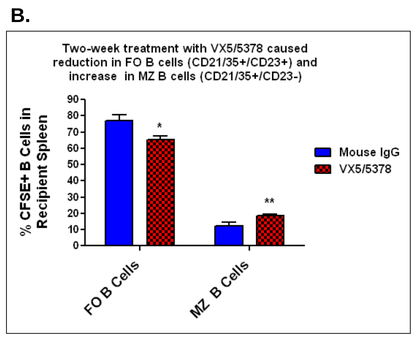

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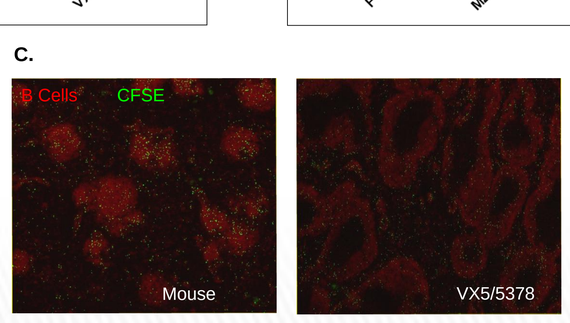
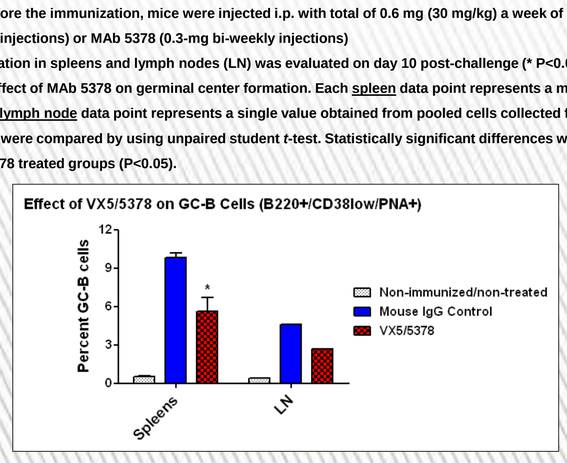


- **Immunized BALB/c Mice:**
  - **BALB/C mice immunized with 100 ug 4-hydroxy-3-nitrophenylacetyl-chicken-g-globulin (NP-CGG) precipitated in 100 ul of alum.**
  - **Starting one week before the immunization, mice were injected i.p. with total of 0.6 mg (30 mg/kg) a week of either Mouse isotype control (0.6-mg weekly injections) or MAb 5378 (0.3-mg bi-weekly injections)**
  - **Germinal center formation in spleens and lymph nodes (LN) was evaluated on day 10 post-challenge (* P<0.05)**
  - **Figure on the right: Effect of MAb 5378 on germinal center formation. Each spleen data point represents a mean of values measured from three mice. Each lymph node data point represents a single value obtained from pooled cells collected from three mice.**

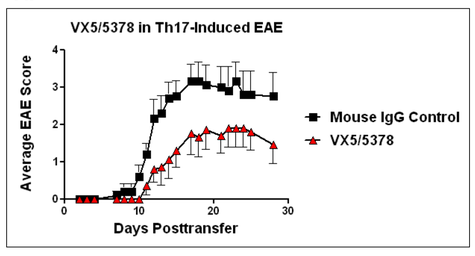
"Spleen" group means were compared by using unpaired student *t*-test. Statistically significant differences were observed between Mouse IgG and MAb 5378 treated groups (P<0.05).



VX5/5378 Reduces Severity of Passive Th17-Induced EAE in SJL/J Mice

- **Rationale**
  - **Human tonsillar, peripheral blood and cord blood Th17 cells (**Singh et al *J. Immunol*. 2008;180;214-221**) and mouse splenic and Peyer Patches' Th17 cells (**Wang et al *Mucosal Immunol*. 2009 March ; 2(2): 173–183**) express CXCR5 receptor.**
  - **Human Th17 cells migrate toward CXCL13 (**Lim et al *The Journal of Immunology*, 2008, 180: 122–129**)**
  - **In Passive EAE (**Jager et al: Th1, Th17, and Th9 effector cells induce Experimental Autoimmune Encephalomyelitis with different pathological phenotypes. *J.Immunol.* 2009; 183; 7169-7177**): "The recipients of 2 Th17 plus IL-23 cells had massive meningeal and parenchymal infiltrates and had almost twice as many lesions as 2 Th1 cell recipients"**
- **Study Design**
  - **Th17 and Th1 subsets were differentiated (in the presence of irradiated splenocytes and 20 ug/ml PLP139-151) from the CD4+CD62L^{high}T cells of donors immunized with PLP139-151 in 1mg/ml CFA enhanced with 5mg of heat-inactivated *Mycobacterium tuberculosis* strain H37RA:**
  - **Th17 differentiation: two days with 30 ng/ml IL-6 , 3 ng/ml huTGF-β, 20 ug/ml anti-muIFN-γ, 20 ug/ml anti-muIL-4; four days with 10 ng/ml muIL-23; 2 days with 1 ug/ml anti-CD3 and 0.5 ug/ml anti-CD28**
  - **Th1 differentiation: two days with 20 ug/ml anti-muIL-4 and 10 ng/ml muIL-12; four days with 20 U/ml muIL-2; 2 days with 1 ug/ml anti-CD3 and 0.5 ug/ml anti-CD28**
  - **EAE was induced in recipient SJL/J mice by i.p. injection of 2X10^6 of donor Th17 or Th1 cells**
  - **Treatment consisted of bi-weekly intraperitoneal injections of 0.6 mg (30 mg/kg) of mouse anti-human CXCL13 antibody (VX5/5378) or irrelevant Mouse IgG control**

A.



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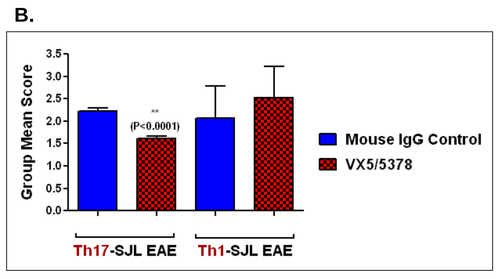

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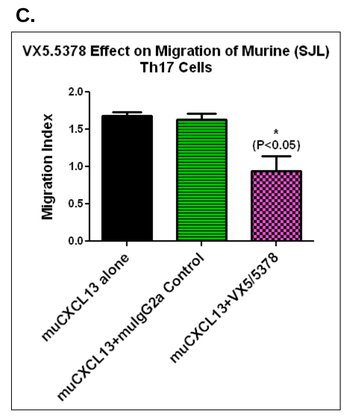


A. **Vx5/5378 in Th17-SJL EAE: Results of a representative experiment.**

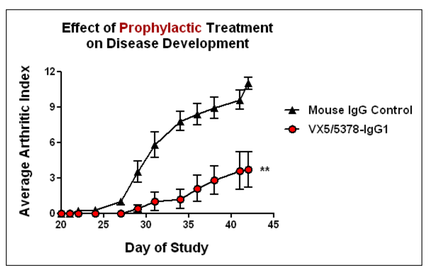
B. **VX5/5378 in Th17 vs. Th1 SJL-EAE: Th17 EAE: GMS +/- SE (GMS calculated from three independent experiments ; group means were compared using unpaired Student's t-test); Th1 EAE: GMS +/- SD: Results of one experiment**

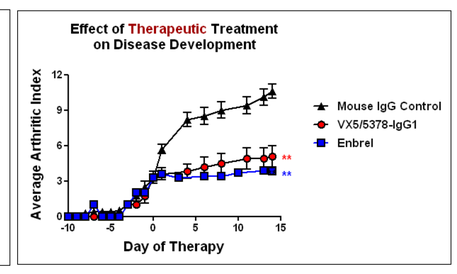
C. **VX5/5378 effect on migration of freshly-thawed Th17 cells from a successful passive EAE transfer. Concentrations: 5 ug/ml muCXCL13; 50 ug/ml of each antibody. Results are presented as average of duplicate measurements +/- SD.**

Confirmation of Anti-Inflammatory Effect: VX5/5378-IgG1 Reduces Severity of Collagen-Induced Arthritis

Study Design

DAY	Procedure
0	Initial, subcutaneous, immunization of DBA1/J mice with bovine Type II collagen in CFA enhanced with heat-inactivated *Mycobacterium tuberculosis* strain H37RA
20	Start Prophylactic Treatment: bi-weekly i.p. injections of 0.6 mg (30 mg/kg) of Mouse IgG Control or IgG1 version of VX5/5378 (VX5/5378-IgG1)
21	Booster subcutaneous injection with bovine Type II collagen in Incomplete Freund's Adjuvant (IFA)
~ 30	Start Therapeutic treatment: bi-weekly i.p. (Mouse IgG control or VX5/5378-IgG1) or s.c. (Enbrel) injections of 0.6 mg (30 mg/kg) antibody starting at group mean Arthritic Index (AI) = 3-4





For each experiment: each data point represents a mean of scores taken from 10 mice. Data were analyzed using unpaired Student's *t*-test (P<0.05).**

Effect of treatment on Joint Histology

	Joint Parameter (Average Score +/- SEM)			
	Inflammation	Pannus Formation	Cartilage Damage	Bone Damage
Prophylactic Treatment				
Mouse IgG Control	4.3 +/- 0.4	1.3 +/- 0.5	2.7 +/- 0.5	1.3 +/- 0.5
VX5/5378-IgG1	1.7 +/- 0.4	0	0.8 +/- 0.1	0
Therapeutic Treatment				
Mouse IgG Control	3.8 +/- 0.3	1.3 +/- 0.5	2.8 +/- 0.7	1.3 +/- 0.5
VX5/5378-IgG1	0	0	0	0
Enbrel	0	0	0	0

Summary

We have generated a mouse multispecies specific (human, mouse and cynomolgus monkey) anti-CXCL13 antibody, 3D2, with demonstrated efficacy in Experimental Autoimmune Encephalomyelitis, a murine model of Multiple Sclerosis.

VX5/5261, a humanized antibody derived from 3D2, shared prototype's multispecies reactivity and demonstrated significantly improved affinity for human CXCL13 and "*in vitro*" functional activity.

Murine version of MAb 5261, MAb 5378, interfered with follicular structure and reduced germinal center formation in naïve and immunized BALB/C mice, respectively; had demonstrated efficacy in Th17-induced EAE in SJL/J mice and therapeutic and prophylactic models of Collagen-Induced Arthritis (CIA) in DBA1/J mice

Master cell bank has been characterized and manufacturing and purification process developed.

Single dose PK study in rats with VX5/5261 has been completed, with no toxicity observed.

VX5/5261 will undergo further development in preparation for an IND submission.