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Anti-CXCL13 and Anti-TNFα Monoclonal Antibodies Combinatorial Treatment Inhibits Autoimmune Disease in a Murine Model of Systemic Lupus Erythematosus

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1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by the involvement of multiple organ systems with alternating clinical exacerbations and remissions. Circulating immune complexes and autoantibodies can cause tissue damage and organ dysfunction with manifestations involving the skin, serosal surfaces, central nervous system, and kidneys (Rahman & Isenberg, 2008).

B cells are believed to play an important role in SLE. B cells can function as APCs, produce cytokines and chemokines contributing to lymphoid regulation, and can respond to stimuli in the microenvironment at local tissues (Ramanujam & Davidson, 2008). Pathogenic autoantibodies produced by autoreactive B cells are believed to play an important role in the pathogenesis of SLE.

CXCL13 has been shown to be a key mediator of organization of lymphoid tissues. CXCL13 is a B cell chemoattractant that is expressed by peritoneal macrophages and follicular dendritic cells in secondary lymphoid organs, such as the follicles of Peyer's patches, the spleen and lymph nodes. Through interaction with CXCR5, a G-protein coupled receptor, CXCL13 attracts B lymphocytes and promotes migration of small numbers of T helper follicular cells and macrophages (Gunn et al., 1998). CXCL13 is critical for B cell homing and follicle formation in lymph node and spleen, and it is required for the development of lymph nodes and Peyer's patches (Ansel et al., 2000). CXCL13 protein level is elevated in ectopic B cell follicles formed in the inflamed tissues of multiple chronic diseases, and plays an important role in maintaining inflammation by actively recruiting B cells (Carlsen et al., 2004; Magliozzi et al., 2004; Salolonsson et al., 2002; Shi et al., 2001;). CXCL13 has been shown to have increased expression in the thymus and kidney of aged NZB/W F1 mice, and may play a role in breaking immune tolerance in the thymus of autoimmune prone mice (Ishikawa et al., 2001). Treatment with anti-CXCL13 has shown efficacy in animal models of RA and EAE (Bagaeva et al. 2006; Zheng et al., 2005). Because of its function and presence in various pathological conditions, CXCL13 and CXCL13 dependent pathways are thought to be instrumental in the pathogenesis of a variety of diseases where B cells may play a significant role, including RA, OA, UC, and SLE, and could be potential targets for autoimmune therapy (Table 1).

Human Disease	Potential role of B cell	Reference
Systemic Lupus Erythematosus	Antibody production, T cell activation, Antigen presentation, cytokine production, lymphoid neogenesis	Lipsky, 2001
Rheumatoid arthritis	Cytokine production, lymphoid neogenesis, T cell activation	Panayi, 2005
Sjogren's Syndrome	Antibody production, lymphoid neogenesis	Liang, 2007
Autoimmune thyroiditis	Antibody production, lymphoid neogenesis	Yu, 2008
Multiple Sclerosis	Lymphoid neogenesis, T cell activation	Hirotani, 2010
Myasthenia Gravis	Lymphoid neogenesis, antibody production	Meraouna, 2006

Table 1. Role of B cells in human autoimmune diseases.

NZB/W F1 mice develop an autoantibody response against DNA and chromatin antigens, a polyclonal hypergammaglobulinemia and ultimately, severe immune complex mediated glomerulonephritis (Aringer & Smolen, 2008). These mice have been widely used as a model to study lupus nephritis. TNFα is a pleiotropic cytokine produced by many cell types that plays a key role in the pathogenesis of multiple autoimmune disorders, as well as a controversial role in SLE (Aringer & Smolen, 2008; Kollias, 1999).

Although individual therapies with anti-TNFα or anti-CXCL13 mAb for additional inflammatory diseases have been explored with limited success, there has not been any attempt to combine the two mabs for the treatment of any disease (Bagaeva et al., 2006; Dick et al., 1996; Ruddle et al., 1990; Zheng et al., 2005). This study was designed to investigate the effect of anti-CXCL13 and anti-TNFα mAbs treatment on disease development in NZB/W F1 mice.

2. Materials and methods

2.1 Antibodies and reagents

RPMI media, heat-inactivated fetal bovine serum, gentamycin and L-glutamine were purchased from Invitrogen (Carlsbad, CA). Neutralizing rat anti-CXCL13 mAb (MAB4701) was purchased from R&D Systems (Minneapolis, MN) with an endotoxin level of 1.2 EU/mg. Anti-TNFα was made at Centocor, and had an endotoxin level of 0.262 EU/mg.

2.2 Animals and experimental protocol

NZB/W F_1 mice aged 10-12 weeks were obtained from Jackson Laboratories (Bar Harbor, ME). On day 0, the study animals were randomly assigned to control or treatment groups (n = 15/group). An intraperitoneal injection of saline, anti-mCXCL13 mAb (0.5 mg/mouse, 2 times a week, weeks 16-34), anti-TNFα mAb (0.5 mg/mouse, 2 times a week, weeks 16-18, then 0.25 mg/mouse, 2 times a week, weeks 19-34) or a combination of anti-CXCL13 plus anti-TNFα mabs were administered weekly from 16 to 34 weeks of age. Animal were monitored weekly. Urine was collected via free catch (once every 3 weeks starting from 12 weeks of age) and stored at -80°. Blood was collected every three weeks starting from 16 weeks of age, and serum was stored at -80°. At the final harvest, spleen, lymph nodes, and kidneys were harvested into appropriate storage buffers before further analysis by in vitro functional assays. This study protocol was reviewed and approved by Centocor's Institutional Animal Care and use Committee.

2.3 Flow cytometry analysis of B cell activation status

Mice were killed at 34 weeks of age and their spleens were removed. A portion of the spleen was placed in cold RPMI-1640 medium supplemented with 10% fetal bovine serum, 10 mg/ml gentamycin, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol. Red blood cells were lysed in red blood cell lysing buffer (Biowhittaker) on ice for 5 minutes. Splenocytes were stained with optimal concentrations of fluorochrome conjugated mAbs (5×10^5 cells in 200 µl of phosphate buffered saline, 1% bovine serum albumin, 0.1% sodium azide) in U-shaped microtiter plates at 4° C for 30 min, and fixed with 1% paraformaldehyde. Samples were analyzed on a FACSCalibur Instrument (Becton Dickinson, Mountain View, CA). Anti-murine CD23 PE (clone B3B4) and anti-murine CD24 FITC (clone M1/69) were purchased from BD Biosciences (Chicago, IL) and used for analysis of B cell activation.

2.4 Autoantibody analysis

Anti-dsDNA autoantibodies were determined by ELISA. Double stranded-DNA coated plates were purchased from DiaSorin (Stillwater, MN). 1:100 diluted serum samples were incubated at room temperature for 2 hours on the plates. Alkaline phosphatase conjugated anti-murine IgG (Southern Biotechnology Associates, Birmingham, AL) was added to the plate for 1 hour followed by incubation with p-nitrophenylphosphate substrate (Sigma, St. Louis, MO) for 30 minutes and the plates were read at OD405 nm. OD values from separate assays were normalized to a single MRL lpr/lpr MRL/MpJ-Fas^{lpr}/J positive control serum.

2.5 Proliferation assays

B cell proliferation was assessed using 1×10^6 splenocytes stimulated with 2 µg/ml each of anti IgM F(ab') (Pierce Biotechnology) and 5 µg/ml anti-CD40 (BD Pharmingen, Sacramento CA) for 72 hours. Proliferation was assessed using BrDU (Roche Applied Science, Indianapolis, IN) and counting luminescence singles on a TopCount (PerkinElmer, Shelton, CT).

2.6 Urine total protein/creatinine analysis

Urine samples were collected from mice via free catch and frozen at -80° C for subsequent analysis of urine total protein/creatinine ratio determined by Ace Analyzer (Alpha Wasserman, West Caldwell, NJ). Urine total protein was measured in undiluted urine and creatinine was measured using urine diluted 1:10 in deionized distilled H_2O.

2.7 Histologic analysis of kidney pathology

Kidneys were harvested and immediately immersed in 0.7% periodate lysine paraformaldehyde (PLP) buffer, composed of 0.1 M phosphate buffer, 0.7% paraformaldehyde, 75 mM L-lysine and 10 mM $NaIO_4$. The kidneys were processed for microscopic examination and embedded in paraffin by routine methods after overnight fixation in PLP buffer. The 5 µm thick sections were stained with haematoxylin & eosin (H&E) for general morphology. Samples were examined and scored for disease severity in a blinded fashion. Pathology was assessed using the World Health Organization (WHO) classifications (Weening et al., 2004).

2.8 Immunohistochemical staining

Spleens were harvested, cut in half along its vertical axis, and one half was suspended in OCT and frozen in 2-methyl-butane cooled with dry ice. Spleen sections were prepared,

fixed in acetone and incubated in PBS (no azide), then in 0.3% H_2O_2 to quench endogenous peroxidase activity. The sections were blocked using PBS/5% normal goat serum/0.1% Tween 20 and stained with biotinylated peanut agglutinin (Vector Labs) and B220 FITC (BD Biosciences). Streptavidin-Horseradish peroxidase (HRP, Southen Biotchnologies) and anti-FITC-alkaline phosphatase (AP, Southern Biotechnologies) were used as secondary antibodies. HRP and AP were developed using 3-amino-9-ethyl-carbazole and Fast-Blue BB base (Sigma Chemical Co., St. Louis, MO) respectively. Samples were examined in a blinded fashion.

2.9 Chemotaxis of purified B cells

B cells were purified by negative selection using the B cell isolation kit from Miltenyi Biotec (Auburn CA). B cell purity was determined by staining for CD19-positive cells and was >95%. Purified murine B cells (4 x 10^7 cells in 10 ml RPMI/10% FBS) were loaded with calcein dye (1 mg/ml in dry DMSO, Molecular Probes, Invitrogen) for one hour at 37° C. Cells were centrifuged at 1200 rpm for 7 min, then resuspended in PBS/2%FBS to a final concentration of 1 x 10^6 cells/ml. CXCL13 (R&D Systems, Minneapolis, MN) was diluted in PBS/2%FBS to a final concentration of 750 ng/ml and aliquoted to a 5 µm Neuroprobe (Neuroprobe, Gaithersburg, MD) 96 well chemotaxis apparatus, and 50 µl of cells were loaded onto the filter. The chemotaxis plate was incubated for one hour at 37° C, then washed and centrifuged briefly to bring the cells to the bottom of the well. Fluorescence at the bottom of the well was read on the Tecan (Tecan, Mannedorf, Switzerland).

2.10 Statistical analysis

Cell surface marker expression, anti-dsDNA levels, B cell proliferation and chemotaxis were expressed as mean ± SE and statistical significance was determined by two tailed analysis of variance by standard t test. For statistical analysis on kidney pathologies, the incidence of severe disease was compared across groups by Fisher Exact test with a Bonferroni adjustment of the nominal type I error to determine the variance among the treatment groups. Rank order histological data was analyzed by ANOVA with Dunn's correction for multiple comparisons. p values < 0.05 were accepted as significant.

3. Results

3.1 Anti-CXCL13/Anti-TNFα treatment increased follicular B cell and reduced transitional B cells in spleen

We first examined the phenotype of B cells harvested from the treated mice. Spontaneous autoreactive B cell development occurs in NZB/W F1 mice with decreasing follicular B cells and increasing transitional B cells over time. At 34 weeks, follicular B cells in mice treated with anti-CXCL13/anti-TNFα mAbs were significantly increased as compared to that in mice treated with saline, (Fig. 1), while transitional B cells were significantly decreased (Fig. 2) by treatment with anti-CXCL13/anti-TNFα mAbs. These observations suggested that anti-CXCL13/anti-TNFα mAbs treatment helps to maintain a relatively normal B cell repertoire in NZB/W F1 mice, potentially interfering with the spontaneous autoreactive B cell development.

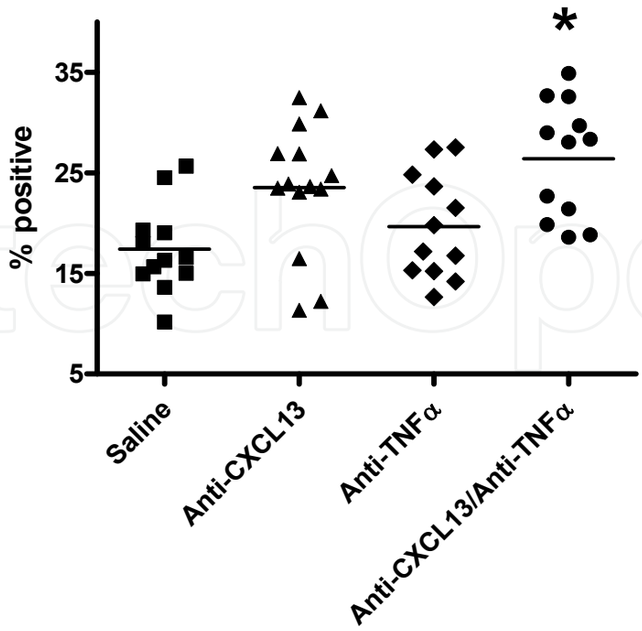

Fig. 1. Anti-CXCL13/anti-TNFα mAbs treatment increased the number of follicular B cells. Total splenocytes were gated on CD19+ B cells and were analyzed with anti-CD23 and anti-CD24 antibodies by flow cytometry to determine the population of CD23+CD24- follicular B cells at 34 weeks of age.. * indicates $p<0.05$ vs saline treated control.

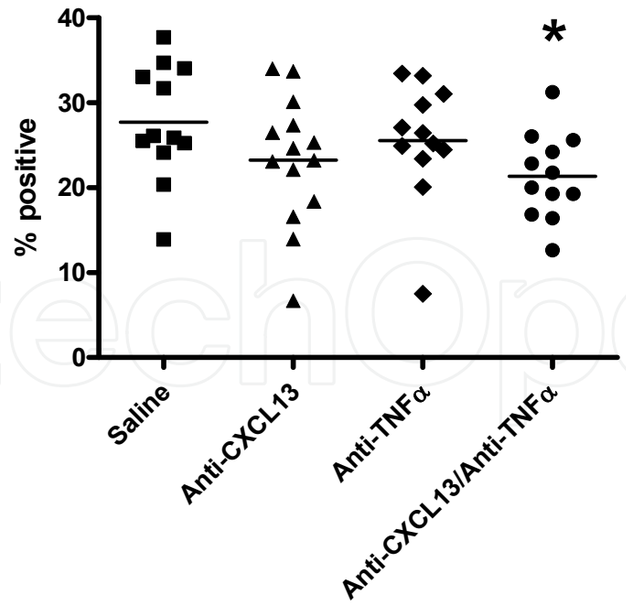

Fig. 2. Anti-CXCL13/anti-TNFα mAbs treatment decreased the number of transitional B cells. Total splenocytes were gated on CD19+ B cells and were analyzed with anti-CD23 and anti-CD24 antibodies by flow cytometry to determine the population of CD23-CD24+ transitional B cells (b) at 34 weeks of age. * indicates $p<0.05$ vs saline treated control.

3.2 Anti-CXCL13/Anti-TNFα mAb treatment inhibited anti-dsDNA autoantibody production in the serum

Since the presence of autoantibodies against dsDNA is a marker of SLE, the effect of anti-CXCL13/anti-TNFα mAbs treatment on anti-dsDNA autoantibody production was examined in the serum samples (Fig. 3). Serum anti-dsDNA autoantibody levels increased over the course of the study, and anti-TNFα or anti-CXCL13 mAb treatment alone did not significantly affect the overall anti-dsDNA production as compared to the control treatment with saline. However, anti-dsDNA production in the animals receiving the combination of anti-CXCL13/anti-TNFα mAbs was significantly decreased as compared to the anti-TNFα treatment group (Fig. 3).

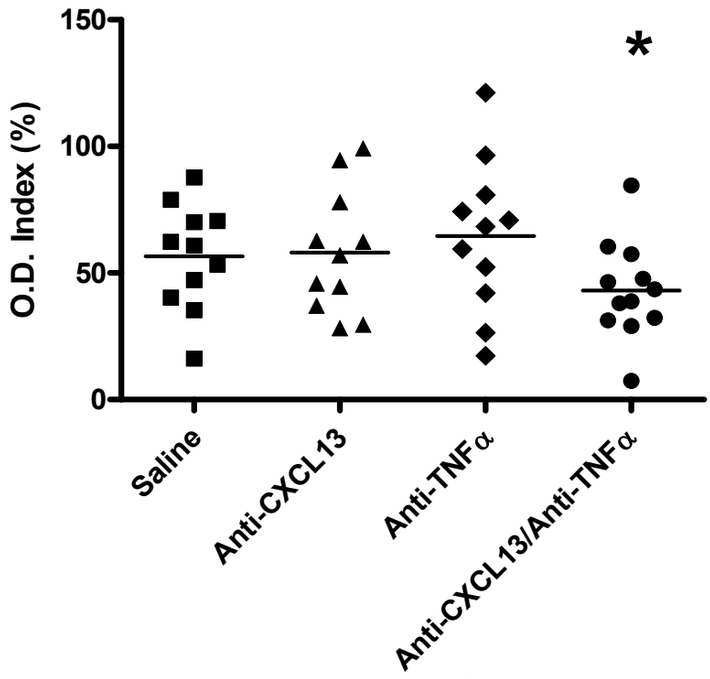

Fig. 3. Anti-CXCL13/anti-TNFα mAbs treatment significantly inhibited serum anti-dsDNA autoantibody as compared to anti-TNFα treatment. Serum samples were analyzed for anti-dsDNA autoantibody levels by ELISA at 34 weeks of age. O.D. index values represent individual data point normalized throughout the studies to a single positive control serum with anti dsDNA. * indicates $p < 0.05$ vs. anti-TNFα mAb treated group.

3.3 Anti-CXCL13/Anti-TNFα mAb treatment decreased B cell proliferation

To further investigate whether anti-CXCL13/anti-TNFα mAbs treatment affects the functions of B cells, antibody induced in vitro proliferation was performed to determine B-cell responses using splenocytes isolated from various treatment groups. Ex vivo B-cell proliferation stimulated with anti-CD40/anti-IgM mAbs was significantly depressed by in vivo anti-CXCL13/anti-TNFα mAbs treatment as compared to the saline and single anti-TNFα or anti-CXCL13 antibody treatments when mice were 34 weeks old (Fig. 4). These

data demonstrated that B cells from animals treated with anti-CXCL13/anti-TNFα mAbs were more resistant to ex vivo stimulation and activation.

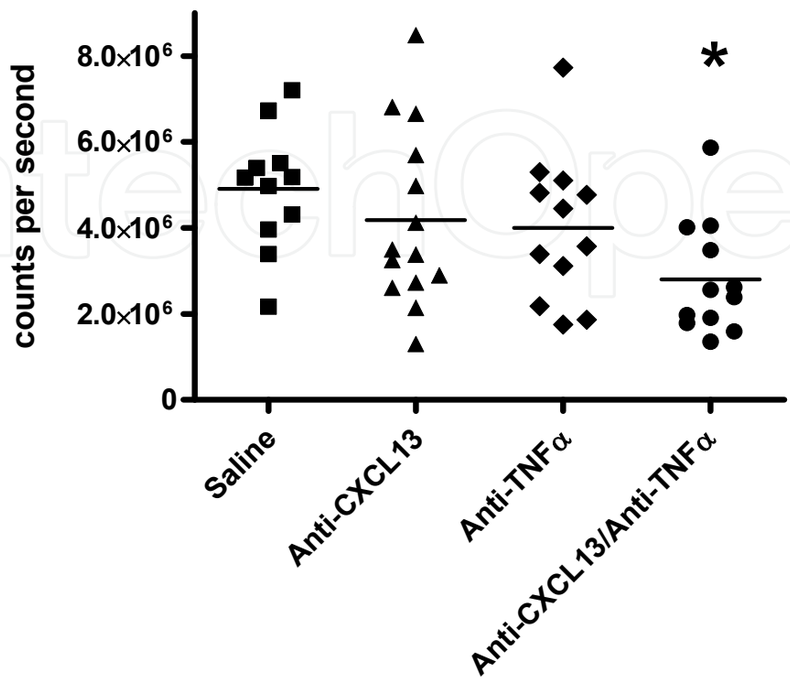

Fig. 4. B cell proliferation is decreased in NZB/W mice treated with anti-CXCL13/anti-TNFα mAbs. Cell Proliferation ELISA using BrDU was performed to determine B cell proliferation and results are expressed as counts per second. * indicates $p<0.05$ vs saline control treated groups.

3.4 Anti-CXCL13/Anti-TNFα mAb treatment suppressed kidney pathology

Glomerulonephritis is another feature of SLE. To determine the effects of anti-CXCL13/anti-TNFα mAbs treatment on kidney function and pathology, we examined urine total protein/creatinine ratios and renal histopathology. Treatment with anti-CXCL13/anti-TNFα mAbs significantly decreased urine total protein/creatinine ratios compared to the anti-TNFα, anti-CXCL13, or saline treatment groups (Fig.5).

At 34 weeks of age, periarterial lymphocytic infiltration at the hilus and along the major branches of the renal artery was observed in the PBS control group. There was also evidence of glomerular disease characterized by an increase in mesangial cellularity, collapse of capillary lumina, thickened basement membranes and the presence of amorphous hyaline deposits. These histological changes were associated with an increase in urinary total protein/creatinine ratio (Fig. 5).

Anti-TNFα or anti-CXCL13 mAb treatment alone did not significantly affect the glomerular disease development at week 34 as compared to the control treatment with saline. The beneficial effect of anti-CXCL13/anti-TNFα mAbs treatment on decreasing renal disease severity was reflected by the rank score of disease severity across the groups for glomerular disease (Fig. 6).

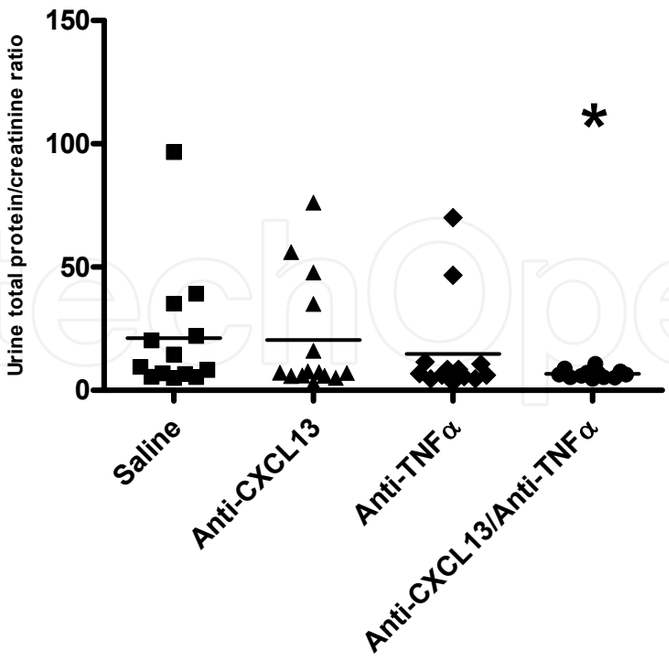

Fig. 5. Anti-CXCL13/anti-TNFα mAbs treatment significantly inhibited urine total protein/creatinine ratios. Urine total protein/creatinine ratios were determined at 34 weeks. * indicates $p<0.05$ vs. saline control treated group.

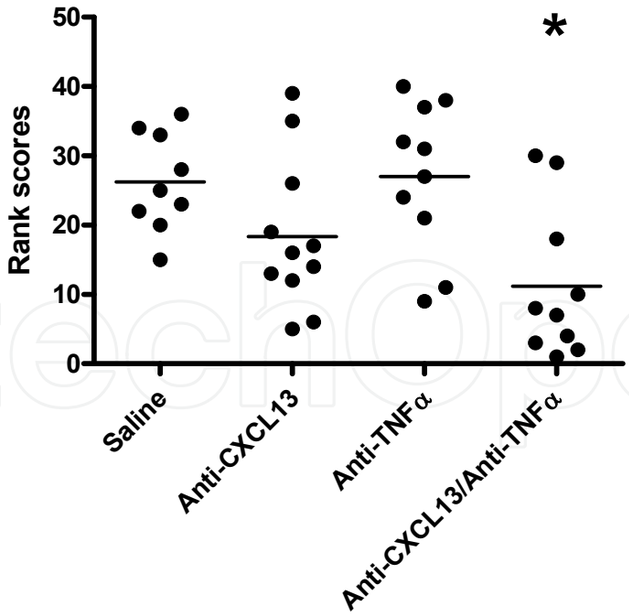

Fig. 6. Anti-CXCL13/anti-TNFα mAbs treatment reduced kidney disease in NZB/W F1 mice. Samples were examined and scored for disease severity in a blinded fashion. Pathology was assessed using the WHO Classifications. * indicates $p<0.05$ vs. saline control treated group.

3.5 Anti-CXCL13/Anti-TNFα mAb treatment decreased germinal center formation

In the splenic germinal center, B cell activation is triggered by ligation with sufficient antigen that has been captured by follicular dendritic cells in a complement and antibody-dependent process. B cell activation leads to migration of B cells towards the T cell zone. B cells then receive help from primed T-helper cells also expressing CXCR5 to form follicles and propagate GCs (Fazilleau et al., 2009). In the GCs, immunoglobulin class switching and somatic hypermutation as well as subsequent selection of centrocytes expressing BCR of increased affinity and specificity for the antigen result in the generation of affinity matured, long-lived plasma cells and memory cells.

To investigate the mechanism by which anti-CXCL13/anti-TNFα mAbs treatment has suppressed autoimmune responses in murine SLE, we examined the spleens for germinal center formation. Immunohistochemical staining for germinal center formation reveals that NZB/W mice treated with anti-CXCL13/anti-TNFα mAbs have decreased germinal center formation (Fig. 7). The reduction of the germinal center formation most likely resulted in a decrease of B cell stimulation and activation which subsequently led to suppressed anti-dsDNA autoAb production and glomerular disease development in mice treated with the anti-CXCL13/anti-TNFα mAbs. Mice treated with either mAb alone had germinal center formation similar to that of the saline treated mice in both number and size.

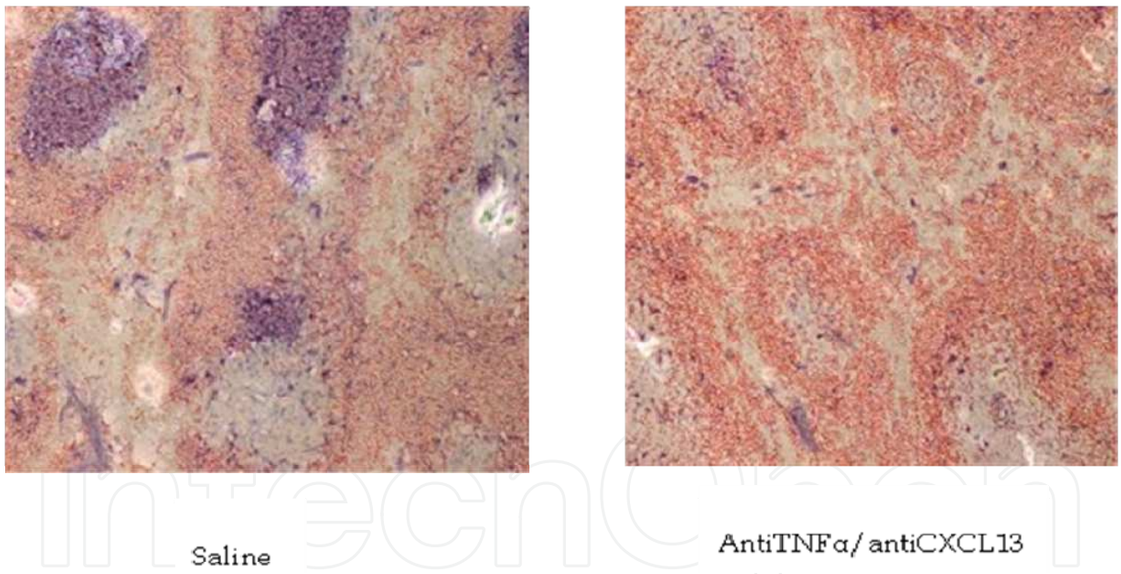

Fig. 7. Anti-CXCL13/anti-TNFα mAbs treatment decreased germinal center formation in NZB/W mouse spleen. (a) Saline or (b) Anti-CXCL13/anti-TNFα mAb treated spleen sections were stained with peanut agglutinin (blue) and anti-B220 (red) to identify germinal center and B cell zones. (Original magnification 20X).

3.6 Anti-CXCL13/Anti-TNFα treatment increased chemotactic activity of naive B cells

By treating NZB/W F1 mice with anti-CXCL13/anti-TNFα mAbs, we were able to inhibit autoimmune disease progression in NZB/W F1 mice. CXCL13 has been shown to be a very specific mature B cell chemoattractant. Expression of CXCR5 in mature naïve B cells is high, but after activation and differentiation, B cells lose CXCR5 expression (Hargreaves et al.,

2001). Thus naïve mature B cells would be more responsive to chemotatic migration induced by CXCL13. We investigated the effect of antibody treatment on B cell chemotaxis in our study. After treatment with anti-TNFα alone or the combination treatment of anti-CXCL13/anti-TNFα mAbs, the B cells purified from splenocytes were significantly more responsive to in vitro chemotactic stimulation induced by CXCL13 as compared to the B cells from animals treated with saline, or anti-CXCL13 mAb alone (Fig. 8).

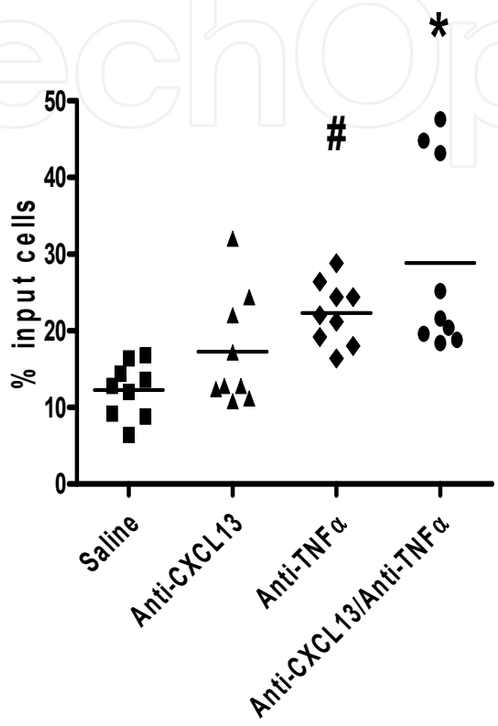

Fig. 8. Anti-CXCL13/anti-TNFα or anti-TNFα mAbs treatment significantly increased chemotaxis of B cells. B cells purified by negative selection over an AutoMacs column were loaded onto a 5 μM 96 well chemotaxis apparatus and exposed to 750 ng/ml CXCL13 for one hour. The cells in the bottom well were counted and expressed as a fraction of the cells loaded onto the apparatus. * and # indicates $p<0.05$ vs saline treated control.

A logical explanation for this observation is that in the saline or anti-CXCL13 mAb treated mice, there are an increased number of activated and differentiated B cells and decreased number of naïve B cells. Activated or differentiated B cells express fewer CXCR5 receptors and thus responded poorly in the chemotaxis assay. In contrast, there are more naïve B cells, which have normal expression of CXCR5 receptors, in the anti-TNFα and anti-CXCL13/anti-TNFα mAbs treated mice. This result is highly consistent with the B cell phenotype described earlier.

4. Discussion and conclusion

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by hyperactivity of autoreactive T and B cell responses against a variety of organs and can have widely varying degrees of severity(Ardoin & Pisetsky, 2008). Traditional therapies include steroids, mycophenolate, azathioprine, cyclophosphamide and hydroxychloroquine, which

utilize various mechanisms of action resulting in global immune suppression and significant side effects (Wallace & Hahn, 2007).

There is a pressing need in the lupus field to find efficacious drugs with more specific immunosuppression. It has been shown by many investigators that various chemokines and cytokines play a role in the progression and pathogenesis of this complex disease (Dorner et al., 2009). It is generally accepted that treatments that would inhibit specific immune cell functions that are responsible for development of SLE may be beneficial for patients. The current study was designed to investigate the effect and mechanism of simultaneous application of two antibodies specific for B cells and inflammation in the inhibition of disease development in a murine model of lupus.

This study shows novel findings that can have applications for potential treatment of autoimmune disease. TNFα is increased in the blood and inflamed kidneys of SLE patients and correlates with disease activity (Ernandez & Mayadas, 2009; Studnicka-Bencke et al., 1996). TNF expression was also shown to be increased in aged NZB/W mice (Shiffer et al., 2008; Studnicka-Bencke et al., 1996). However, other literature addressing the role of TNFα in SLE suggests that it has a complex function. Administration of TNFα reduces incidence of SLE in young NZB/W F1 mice (Jacob et al., 1991). In NZB/W F1 mice, TNFα deficiency accelerates autoimmune disease and the mice develop severe lupus-like disease including autoantibodies to dsDNA and immune complex glomerulonephritis (Aringer & Smolen, 2008). TNFα seems to check autoimmunity in some paradigms, and foster inflammation in others, suggesting that other factors not yet identified may contribute to the role played by TNFα in SLE. This actually in part accounts for why we did not observe significant inhibition of autoimmune responses by anti-TNFα treatment alone in the current study.

Anti-dsDNA autoantibody levels in the serum were sometimes associated with disease activity and immune complex formation as well as glomerulonephritis in patients and mice. In our study, treatment with anti-CXCL13/anti-TNFα mAbs resulted in decreased anti-dsDNA autoantibody levels in the serum of NZB/W F1 mice, as compared with that of the TNFα alone treated mice (Fig 3). This result showed that blocking TNFα alone is not enough to suppress the autoimmune responses in this model as it did in other models. The likely reason could be the heavy involvement of B cells in such responses. The combination therapy with blockade of both TNFα and CXCL13 is superior to just the TNFα blockade alone due probably to the simultaneous suppression of both autoreactive B cells and TNFα.

CXCL13 participates in the follicular compartmentalization of B cells in GC and the induction of lymphotoxin ($LT\alpha_1\beta_2$) expression on B cells (Ansel et al., 2000). GCs support the differentiation of memory B cells and long-lived antibody secreting plasma cells. CXCL13 plays an important role in attracting naïve B cells to form germinal centers and can initiate lymphoid neogenesis when expressed aberrantly in mice (Cyster, 1999; Melchers et al., 1999; Takemura et al., 2001). Ectopic CXCL13 was expressed in aged NZB/w mice developing lupus nephritis (Ito et al., 2004). CXCL13 was enhanced in the thymus and kidney of aged NZB/w F1 mice (Ishikawa et al., 2001). There was a decreased number of CXCL13 producing peritoneal macrophages in aged NZB/w mice and the ectopic high expression of CXCL13 results in abnormal B1 cell trafficking during the development of murine lupus (Ito et al., 2004). As expected, treatment of NZB/w mice with a combination of anti-CXCL13 and anti-TNFα mAbs resulted in decreased germinal center formation in spleen sections in our study (Fig. 7). Combined treatment with anti-CXCL13/anti-TNFα mAbs significantly inhibited ex vivo IgM/CD40 stimulated B cells proliferation (Fig. 4), increased the

frequency of follicular B cells (Fig. 1), and decreased the frequency of transitional B cells in the spleen (Fig. 2), when the total spleen cell number was not changed (data not shown) in our study. These novel results demonstrate that the combination therapy significantly dampens the autoimmune response in this model by maintaining a relatively normal lymphoid structure as well as B cell repertoire and lowering the activation status of the B cells, resulting in a higher threshold for hypereactivity.

Glomerulonephritis is a consequence of immune complex deposition and subsequent inflammatory cell infiltration and is a pathological hallmark feature of murine SLE. TNFα is highly expressed in glomeruli in all forms of lupus nephritis and the degree of TNFα expression correlates with renal inflammatory activity (Aringer & Smolen, 2003; Herrerra-Esparza et al., 1998). Administration of anti-CXCL13/anti-TNFα mAbs in our study significantly decreased the disease severity of glomerulonephritis in NZB/w F1 mice (Fig. 5 & 6), as reflected in decreased protein/creatinine ratios and kidney disease scores. In addition to the impact on B cells, neutralization of TNFα and CXCL13 could also result in decreased DC recruitment in the circulation and decreased DC differentiation and maturation into CXCL13 producing DC which has been suggested to play a pivotal role in the development of SLE (Ishikawa, 2002). In addition, treatment of NZB/W F1 mice with anti-TNFα or a combination of anti-CXCL13/anti-TNFα mAbs in our study resulted in a significant increase of mature B cell chemotactic response mediated by CXCL13. In the saline treated group, there were a large number of activated and differentiated B cells in the spleen, which do not express CXCR5 and therefore cannot respond to CXCL13 mediated chemotaxis. Treatment with anti-TNFα or anti-CXCL13 mAb alone did not result in significant inhibition of autoimmune responses and kidney nephritis in this particular murine lupus model. Treatment with anti-CXCL13 only affects naive mature B cell migration to the germinal center. The activated and memory B cells that contribute significantly to the autoimmune responses and disease development in this animal model were not significantly impacted by the anti-CXCL13 mAb, which limited subsequent efficacy. Furthermore, TNFα can interact and signal through two different receptors: TNFR1 and TNFR2, which can also bind LTα. LTα links with two LTβ molecules to form a heterotrimer that signals through LTβR (Browning et al., 1997). Both of these receptor pathways have been shown to activate expression of many genes, including CXCL13 (Ngo et al., 1999). Treatment with anti-TNFα mAb alone may only block the biologic activity of TNFα in symptoms driven by chronic inflammation, but not necessarily the autoimmune responses mediated by autoreactive B cells and LTβR with LTα. LTα would still be able to signal through TNFR or LTβR and contribute to increased CXCL13 expression and enhanced chemotaxis which may account for normal GC formation in the spleen of the TNFα mAb alone treated mice in our study. Simultaneously blocking both TNFα and CXCL13 allowed interruption of complementary inflammatory pathways, suppressed CXCL13 production and FDC maturation that contributes to the ultimate autoimmune disease development in this murine lupus model.

Further characterization of the effect of neutralization of CXCL13 and TNFα in this disease model might be achieved by use of an anti-CXCL13 antibody with increased potency, to ensure complete neutralization of CXCL13. Also neutralization of LT to evaluate the complete shutdown of the TNFα signaling pathway on the development of disease would be useful to characterize its contribution to disease development. An investigation of the

effect of the combination of anti-CXCL13 and anti-TNFα in a therapeutic format could determine pathways essential in established disease. Additional studies to further characterize the mechanism of action of anti-CXCL13/anti-TNFα combinatorial treatment could include the contribution of cells from the innate immune system. Specifically, findings have been linked to mast cell stabilization including normalization of the B cell antibody profile for the promotion of innate as well as adaptive immunity during developmental phases of inflammation-induced immune dysfunction (Khatami, 2008, 2011)

In conclusion, this study demonstrated that combined administration of anti-TNFα and anti-CXCL13 mAbs significantly inhibited autoimmune responses and autoimmune disease progression in the NZB/W F1 murine model of systemic lupus erythematosus. This combined therapy could provide added benefit for advanced lupus patients that have advanced autoimmune disease.

5. Acknowledgements

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6. References

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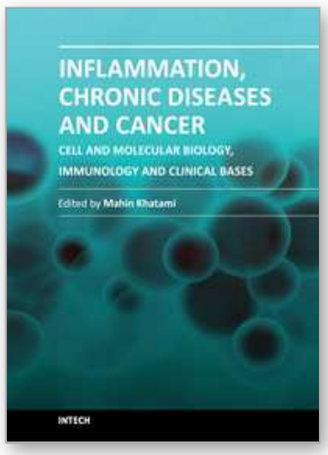

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This book is a collection of excellent reviews and perspectives contributed by experts in the multidisciplinary field of basic science, clinical studies and treatment options for a wide range of acute and chronic inflammatory diseases or cancer. The goal has been to demonstrate that persistent or chronic (unresolved or subclinical) inflammation is a common denominator in the genesis, progression and manifestation of many illnesses and/or cancers, particularly during the aging process. Understanding the fundamental basis of shared and interrelated immunological features of unresolved inflammation in initiation and progression of chronic diseases or cancer are expected to hold real promises when the designs of cost-effective strategies are considered for diagnosis, prevention or treatment of a number of age-associated illnesses such as autoimmune and neurodegenerative diseases as well as many cancers.

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