

Abstract 43 Figure 1

splenocytes (effector) were incubated with K-562 (target) in a ratio of 100:1 for 4 hour once a week until the end of the experiment.

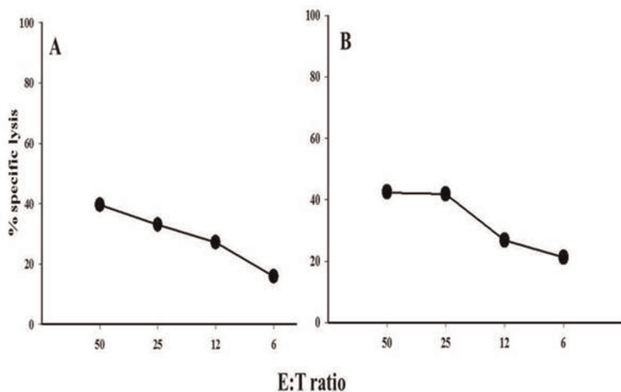
Conclusions The splenocytes from KRG treated porcine showed a significantly increased cytotoxicity in time dependent fashion. Whereas, splenocytes from untreated porcine showed a less toxicity. Taken together, KRG has the potential to modulate immune function and should be further investigated as an immunomodulatory agent.

44 GASTRIC CANCER CELL MICRO ENVIRONMENT MODULATES THE NK CELL EFFICACY IN RAT SPLENYOTES

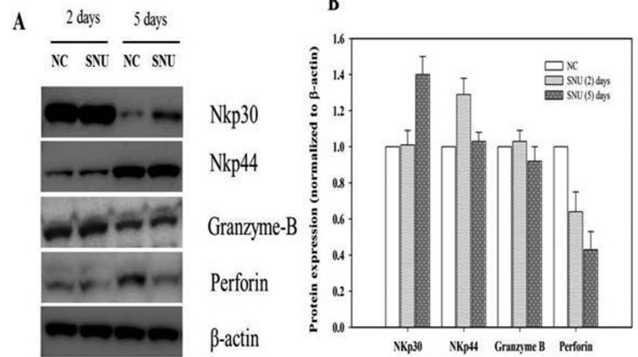
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Background and aims Natural killer (NK) cells are specialised lymphocytes capable of counteracting pathogens (bacteria, viruses) as well as cancer cells. Unlike T lymphocytes, NK cells do not require antigen-specific recognition to act on target cells. The activation of NK cell requires the action of certain pro-inflammatory cytokines (IL-2, IL-12, IL-18, IL-21).



Abstract 44 Figure 1 Cytolytic activity of splenocytes against K562 cells at 24 h with various effect to target (E:T) ratio. (A), untreated splenocytes; (B), splenocytes were treated with 1% (v/v) SNU 484 supernatant for 1 day prior to the experiment,



Abstract 44 Figure 2 (A) Western blot analysis of 1% SNU 484 cell supernatant treated splenocytes for NK cell markers; (B), Normalized expression of various NK cell makers compared to β -action.

Methods Gastric cancer cells (SNU-484) were grown in RPMI medium with 10% heat inactivated FBS at a seeding density 1×10^6 cells/mL for 3 days, supernatant was concentrated 10 fold. Splenocytes were treated with 1% (v/v) SNU-484 supernatant for various periods of time.

Results Flow cytometry (FCM) results suggests that the treatment do not affect the viability of the cells during the study period, further the intracellular levels of NKP30, NKP44, granzyme B, perforin were assessed using Real time-PCR (RT-PCR) and western blot techniques. RT-PCR revealed that NK cell markers were initially down-regulated during 2 days of incubation and increased several folds higher during 5th day when compared to normal control. However, no significant changes were observed in protein expression. SNU-484 cells supernatant treated splenocytes were further analysed for cytolytic activity against K562 cell line as a target with varying (1:6, 1:12, 1:25 and 1:50) target to effector ratio for a period of 24 hour.

Conclusions The results suggest that the treated splenocytes have significantly increased cytolytic activity (49.4%) at the lower effector to target ratio (1:25) when compared to untreated control splenocytes (38.2%). Our results indicate that gastric cancer cell micro-environment can modulate the NK cells efficacy to act against cancer.

45 INDUCTION OF DIFFERENTIATION OF REGULATORY T CELLS COUPLED TO ENDOPLASMIC RETICULUM STRESS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background and aims The aim of this study was to investigate the proportion of regulatory T cells (Tregs) in the peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE) compared with that of healthy controls (HCs). We also evaluated the differentiation difference of induced Tregs in vitro under the presence or absence of endoplasmic reticulum (ER) stress, which is one of the causal factors triggering lupus flares.

Methods We isolated the PBMCs of 16 SLE patients and 11 HCs. The percentage of CD4⁺CD25⁺FoxP3⁺ Tregs was

analyzed using flow cytometry. The PBMCs were incubated with anti-CD3/CD28 beads, supplemented with transforming growth factor- β and interleukin-2 to induce differentiation of Tregs, with or without tunicamycin for 36 hours.

Results The percentage of Tregs in the PBMCs of SLE patients was lower than that in the HCs (1.8 ± 0.9 versus $2.6 \pm 0.7\%$, $p=0.02$). The induced differentiation of Tregs increased in both groups, and the increased proportion was greater in the SLE group (600 ± 351 versus $252 \pm 95\%$, $p=0.01$). Incubation with tunicamycin in the Treg differentiation process also increased the proportion of Tregs in both groups (385 ± 259 versus $166 \pm 105\%$, $p=0.006$), and the increased proportion was higher in the SLE group.

Conclusions The baseline percentage of Tregs was lower in SLE patients than in HCs. However, when Treg differentiation was induced, the differentiation of Tregs was more pronounced in the SLE group. This exaggerated differentiation may reflect the paradoxical response to the diminished suppressive capacity of Tregs in SLE patients.

46 CD11c+T-BET+ B CELL IS CRITICAL FOR ANTI-CHROMATIN IGG2A PRODUCTION IN THE DEVELOPMENT OF LUPUS

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Background and aims A hallmark of systemic lupus erythematosus is high titers of circulating autoantibodies. Recently a novel CD11c+ B cell subset has been identified in aged female mice that is critical for the development of autoimmunity. Transfer of MHC II-mismatched splenocytes from Bm12 mice into B6 mice causes a chronic graft versus host reaction (cGVHD), which is characterised by the production of high titers of autoantibodies and immunopathology that closely resemble SLE. The aim of this study was to figure out the role of CD11c+ B cell in the production of autoantibodies during the development of lupus induced by cGVHD.

Methods We developed and validated cGVHD model by splenocytes transfer of Bm12 mice into B6 mice and identified CD11c+ B cell by flow cytometry and examined anti-chromatin antibody by ELISA. We also identified CD11c+T-bet+ B cell of peripheral blood mononuclear cells obtained from SLE patients and healthy controls.

Results CD11c+T-bet+ B cell was significantly increased in the development of lupus induced by cGVHD. CD138+CD11c+ B cell produced large amounts of anti-chromatin IgG2a upon *in vitro* stimulation. Depletion of CD11c+ B cells significantly ameliorated anti-chromatin IgG2a production *in vivo*. T-bet deficiency impaired the expression of CD11c in B cells and anti-chromatin autoantibodies production in the process of cGVHD. The accumulation of T-bet+CD11+ B cell was found in lupus patients.

Conclusions Our data demonstrated the aberrant activation and differentiation of CD11c+T-bet+ B cell, which produced large amounts of anti-chromatin IgG2a in lupus murine model and patients.

47 THE MEMBRANE-CYTOSKELETON LINKER EZRIN AND SRC FAMILY KINASE LYN COLLABORATE TO MAINTAIN OPTIMAL B CELL ACTIVATION AND PREVENT THE DEVELOPMENT OF AUTOIMMUNITY

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Background and aims Systemic lupus erythematosus (SLE) is characterised by hyperactive B cell antigen receptor (BCR) signalling, autoantibody production and glomerulonephritis. Human GWAS studies have shown a strong association between alterations in the Src family kinase Lyn and incidence of SLE. Mice with genetic deletion of Lyn lose peripheral B cell tolerance and display all the hallmark symptoms associated with human SLE. Therefore, Lyn^{-/-} mice represent a clinically relevant model to investigate the molecular regulation of B cell autoimmunity in SLE. We have previously reported that the membrane-cytoskeleton linker protein Ezrin regulates various facets of B cell function through its dynamic phosphorylation and dephosphorylation. Interestingly, we observed that Ezrin is hyperphosphorylated in Lyn^{-/-} B cells, leading to the hypothesis that Ezrin facilitates B cell autoimmunity in Lyn^{-/-} mice.

Methods To test our hypothesis we generated double knockout mice (DKO) bearing systemic deletion of Lyn and conditional deletion of Ezrin in the B cell lineage. B cell activation, lupus-associated autoantibodies and kidney pathology were investigated.

Results Compared to Lyn-deficient mice, the DKO mice displayed reduced germinal centre B cell and plasma cell differentiation, and decrease in autoantibody levels and glomerulonephritis. Further, an increase in BCR repertoire diversity and inhibition of BCR signalling pathways was observed in DKO B cells.

Conclusions Investigation of proteins that drive B cell hyperactivation in SLE is important for the development of effective and novel therapies. Our data demonstrate that ezrin is an important regulator of B cell activation in the absence of Lyn, and thus a potential molecular target in SLE.

48 IXAZOMIB, AN ORAL PROTEASOME INHIBITOR, REDUCES ANTIBODY PRODUCTION BY DEPLETING PLASMA CELLS IN A T CELL DEPENDENT ANTIGEN RESPONSE MODEL

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Background and aims Pathogenic auto-antibodies produced by plasma cells are key drivers of many auto-immune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), and Sjogren's Syndrome (SS). In addition, solid organ transplant rejection is also mediated by antibodies produced against the donor organ. Plasma cells are highly metabolically active antibody factories and thus sensitive to depletion by proteasome inhibitors. Ixazomib, an oral