Current Trends in Immunology
Vol. 20, 2019

Original Article

Role of CD27 in the regulation of Th1 and Th2 responses in T cell subsets

Seetha Tamma* and Keri Wyllie

Department of Biomedical Sciences, School of Health Professions and Nursing, LIU POST, Brookville, NY 11548, USA.

ABSTRACT

The cross-linking of the cell surface molecule by its specific antibody mimics the binding of the ligand to the receptor and leads to the aggregation and clustering of molecules. This study is undertaken to investigate the signal-transducing potential of CD27 and the engagement of CD27 by cross-linking with anti-CD27 antibody. We investigated 1) whether CD27 cross-linking along with CD3 cross-linking would differentially affect different T cell subsets, and 2) whether signals delivered *via* CD3 plus CD27 and CD28 plus CD27 in CD45RA and CD45RO T cells could act synergistically on the activation of transcription factors, cytokine synthesis, proliferation and overall induction of T cell activation. We analyzed the intracellular signaling events and the data showed that co-cross-linking with both co-receptors induces p38 and IL-4 secretion in CD4+CD45RO+ cells, and ERK2 and IFN-γ in CD4+CD45RA+ cells in an antigen-independent manner. Various subsets of T cells were isolated from PBMC by negative selection using Dyanl magnetic beads. Purified cells were stimulated through receptors by cross-linking with antibodies. MAP kinases, STAT6, STAT1, GATA3 and T-bet were analyzed by western blotting. Cytokine profiles by ELISA and cell proliferation by MTT Assay were analyzed. CD3XL+CD27XL and CD28XL+CD27XL induced Th2 phenotype in CD4+CD45RO+ cells and similar cross-linking resulted in Th1 phenotype in CD4+CD45RA+ cells. It is also clear from these studies that p38MAPK, STAT6, and GATA3 appear to be associated with Th2 phenotype, and MAP kinase ERK2, STAT1, T-bet and IFN-γ appear to be associated with Th1 phenotype. We also observed a positive relationship between activation of p38MAPk and IL-4 synthesis by utilizing p38MAP kinase inhibitor SB203580. Our data suggests that CD4+CD45RA+ cells act as TH1 type and CD4+CD45RO+cells act as TH2 type in an antigen-independent manner. We also observed strong T-bet activation in CD8+ T cells in both subsets. Signal transmission and activation through TNFR family members, such as CD27-mediated co-receptor signaling may increase Th1 effector functions and be possibly beneficial in immunity against tumors and viral infections.

KEYWORDS: STAT6, STAT1, GATA3, T-bet.

INTRODUCTION

CD27 is a 120 kDa type I transmembrane protein, member of the TNFR superfamily (TNFRSF7), which is constitutively expressed as a co-stimulatory molecule on Naïve, activated and memory T cells, NK and NKT cells, regulatory T cells and B lymphocytes [1]. In the absence of CD27-mediated signals, peripheral pools of CD4 and CD8 effector lymphocytes are dramatically decreased [2]. Therefore, CD27 similar to CD28, acts in concert with the T cell receptor to support T cell expansion [3-5].

Two distinct signals are required for optimal T cell activation, one of which is transduced through the polymorphic T cell receptor (TCR) upon binding

*Corresponding author: stamma@liu.edu

to its specific peptide ligand presented by the appropriate major histocompatibility complex (MHC) on antigen presenting cells (APC). The second signal provides an independent stimulus that is triggered by ligation of nonpolymorphic cell surface receptors. Interactions between accessory molecules on T cells and their ligands on APC play a key role in regulating T cell effector activity. Therefore, the factors controlling the expression of these molecules are important determinants in the outcome of T cell activation and the immune response. The CD27-CD70 pathway appears to be important in T-B interaction, T cell activation, cell survival, maintenance of memory cell function, anti-tumor cellular immunity and autoimmunity [3-7]. Ligation of CD27 by CD70 appears to be important in T cell activation and T-B interaction. CD27 and CD70 interaction has been shown to result in IgE synthesis since CD27-negative B cells do not produce IgE [6-8]. CD28 knockout mice have been observed to preserve Thl-mediated cytotoxic T lymphocyte and cellular immunity, but impaired Th2-dependent lg production [9]. Thus CD28 signaling may regulate the balance of inflammatory/humoral (Thl/Th2) responses during an immune reaction. CD27 was found to rescue CD28(-/-) T cells from death at the onset of division, explaining its capacity to support a T cell response in the absence of CD28 and or in collaboration with CD28 in T cell function [5, 6].

STAT proteins were originally described as latent cytoplasmic transcription factors that require phosphorylation for nuclear retention. Extracellular binding of cytokines to respective receptors induces activation of the intracellular Janus kinase (JAK) that phosphorylates a specific tyrosine residue in the STAT protein which promotes the dimerization of STAT monomers *via* their SH2 domain. Once inside the nucleus the active STAT dimer binds to cytokine-inducible promoter regions of genes containing gamma activated site (GAS) motif and activates gene transcription. Proteins called importins and exportins play an important role in transporting proteins into the nucleus and exporting them out of nucleus and into the cytoplasm. This function of importins and exportins is regulated by the small Ras-related GTPase, Ran. The STAT protein can be dephosphorylated by nuclear phosphatases which inactivate STAT and the transcription factor is transported out of the nucleus by RanGTP. The STAT6 family is activated *via* JAK1 and JAK3 upon binding of the cytokine IL-4 (ligand) to the IL-4 receptor IL-4Ra. A common gamma chain (γc)-chain results in JAK1 and JAK3 phosphorylation-ultimately resulting in tyrosine phosphorylation of STAT6 [10-11].

GATA3 is a zinc finger transcription factor that is essential for the differentiation of common lymphoid progenitors into early double negative (DN) thymocytes [12]. GATA3 is expressed throughout thymic development. The GATA family consists of six members; GATA1, 2, and 3 are expressed predominantly in hematopoietic cells, and regulate differentiation and gene expression in T-lymphocytes, erythrocytes and megakaryocytes [12], while GATA4, 5 and 6 are predominantly found in the heart and intestines and involved in cardiogenesis and intestinal development [12].

The transcription factor T-box expressed in T cells (T-bet; Tbx21) has been identified as an important determinant of Thl lineage commitment and immune responses, playing critical roles in the establishment and/or maintenance of effector cell responses in T and B lymphocytes, as well as dendritic cells and natural killer cells. Several autoimmune diseases, especially those classically considered related to T helper 1 (Thl) immunity, appear to require T-bet, as observed in some mouse models [13]. T-bet up regulates granzyme B and perforin production by NK cells [14]. As an example, T-bet is up regulated in patients with Crohn's diseases, an inflammatory disorder driven by Thl cytokines. Crohn's patients showed enhanced INF-γ production as well high levels of T-bet expression. T-bet-deficient mice display drastically increased suspectibility for infections with *Mycobacterium tuberculosis* and *Salmonella* species [15, 16].

Our objective was to investigate the signal transducing potential of CD27 and the engagement of CD27 by cross-linking with anti-CD27 antibodies. In this study we analyzed CD27XL along with CD3XL or CD28XL in both CD4+CD45RA+, CD8+CD45RA+ cells and CD4+CD45RO+, CD8+CD45RO+ T cells on Th1 and or Th2 shift based on activation of transcription factors and cytokine production. Our data shows that CD27 treatment alone or CD28 treatment alone induced minimal levels of IL-4 synthesis in CD4+ CD45RA+; however, engagement

of CD28 alone induced IL-4 synthesis in CD4+CD45RO+ T cells.

MATERIALS AND METHODS

Reagents

Purchased materials and reagents for the experiments conducted included the following: CD4, CD45RA and CD45RO beads (Dynal); Anti- CD3 antibody, anti-CD27 and anti-CD28 antibodies from BD Pharmingen (San Jose, CA); Phospho-ERK antibodies, phosphor STAT-6 antibody (Upstate Biotechnology, Lake Placid); phospho-p38 antibody (Cell Signaling Technologies Inc. Beverly, MA); p38 MAPK inhibitor SB203580 (Calbiochem, La Jolla, CA); Antibodies to phosphorylated STAT 1, STAT 4, Phosphorylated STAT 4 (Zymed lab, CA); anti-T-bet Abs (H-210) were from Santa Cruz Biotechnology (Santa Cruz, CA); Kits for IL-4, TNF-α and IFN-γ (Biosource International, Camarillo, CA); MTT Assay kit from Chemicon International Temecula, CA; SDS gradient gels and polyvinylidene difluoride (PVDF) membrane (Biorad, Hercules, CA); Anti-Stat1 (1/Stat1) and anti-phospho-Stat1 (Y701) were obtained from BD Biosciences and Upstate Biotechnology, respectively. ELISA kits from Biosouce (Camarillo, CA) were used.

Isolation and purification of cells

Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteers by Ficoll-Hypaque (Lymphoprep, Nycomed) density gradient centrifugation. Adherent cells were removed by incubation in the petridishes for 2 hours at 37 °C. Various subsets of T cells were purified by negative selection by utilizing Dynal beads (Great Neck, NY). These T cells were further separated into CD45RA+ and CD45RO+ subsets using anti-CD45RA and anti-CD45RO magnetic beads (Dynal, Great Neck, NY) and the purity was 99%. All cell cultures were carried out in complete RPMI 1640 (cRPMI) medium supplemented with 1% penicillin/streptomycin, 1% L-glutamine and 10% heat inactivated fetal calf serum (FCS).

Cell culture

Cell culture set up was as shown in Table 1. CD27-mediated CD45RA+ and CD45RO+ T cell activation was examined by cross-linking CD27 by anti-CD27mAb plus anti-mouse Ig alone or in the presence of the CD3 or CD28 antibody. As a positive control cells were cultured in the presence of CD3 plus CD28 antibodies. For proliferation assay, cells were cultured for 72 hours, for cytokine analysis cells were cultured for 48 hours, and for transcription factor analysis cells were cultured for 24 and 48 hours. Where indicated, cells were pretreated with p38 MAP kinase (SB203580) inhibitor or, for control, with the solvent of the inhibitors (DMSO) for 1 h (45 min at 37 °C and 15 min on ice).

Western blotting

Protein levels of STAT6, STAT1, GATA3 and T-bet were determined using Western blot technique. Following cell cultures by cross-linking with antibodies as indicated, reactions were terminated by washing cells in ice cold phosphate buffered saline (PBS) + EDTA + sodium orthovanadate and lysed in lysis buffer (0.04 mol/L Tris-HCl, 0.276 mol/L NaCl, 20% glycerol, 2% NP40, 0.002 mol/L sodium orthovanadate, 0.02 mol/L NaF, 10 ug/ml Aprotinin, 10 ag/ml Leupeptin, 0.004 mol/L EDTA, and 1 mmol/L Phenylmethylsulfonyl fluoride (PMSF).

Table 1. Experimental design.

Cells		**Culture conditions**				
	Med	**Ant-CD27 10ug/1x10^6 cells**	**Anti-CD28 10ug/1x10^6 cells**	**Antibodies - CD3+CD28 5ug +10ug /1x10^6 cells**	**Antibodies- CD3+CD27 5ug +10ug /1x10^6 cells**	**Antibodies CD28+CD27 5ug +10ug /1x10^6 cells**
CD4+CD45RA+	+	+	+	+	+	+
CD4+CD45RO+	+	+	+	+	+	+
CD8+CD45RA+	+	+	+	+	+	+
CD8+CD45RO+	+	+	+	+	+	+

Lysed samples were micro-centrifuged for 20 minutes at 14,000 RPM at 4 °C. Samples were incubated with Protein G-sepharose beads for 1 hr, centrifuged, and the supernatants were incubated with phospho-antibodies on a rotator for 2 hrs. The immune complexes were immunoprecipitated with protein G-sepharose beads. Samples (50 micrograms/lane) were mixed with sample buffer, analyzed by SDS-PAGE using 4-20% gradient gels (Biorad) followed by immunoblotting with specific Phospho-antibodies (STAT1, 1:500; STAT6, 1:500; T-bet 1:500; GATA-3 1:500; ERK2 and P38) as before [17, 18] followed by HRP-conjugated secondary antibody and detected by ECL system (Amersham).

Enzyme-linked immunosorbent assay (ELISA)

Cells treated as described above were cultured for 48 hours, and culture supernatants were harvested and analyzed for the cytokines IL-4, IFN-γ and TNF-α using commercial kits from Biosource (Camarillo, CA) according to manufacturer's instructions [17, 18].

Lymphoproliferation assay

Cells were stimulated with various stimulants as described above and were cultured for 3 days and proliferation was assessed by MTT assay kit according to manufacturer's instructions.

All experiments were performed in triplicates. Statistical analysis was performed using Student's two-tailed t-test comparing controls and anti-body stimulations of CD45RA+ and CD45RO+ cells.

RESULTS

Cell subsets and experimental design showing cross-linking and co-cross-linking of surface receptors are displayed in Table 1. Phosphorylation events are shown in Figure 1 and Table 2.

Increased phosphorylation of ERK2 is seen in CD4+CD45RA+ and CD8+ CD45RA+ and CD8+45RO+ cells when compared with CD4+CD45RO+ cells. Increased phosphorylation of P38 and STAT6 is seen in CD4+CD45RO+ cells when compared with the others ($p = 0.05$).

STAT1 phosphorylation is lower in CD4+CD45RO+ cells compared with both CD4+CD45RA+ cells as well CD8+CD45RO+ and CD8+CD45RA+ cells ($p = 0.05$). GATA-3 phosphorylation levels are significantly higher in CD4+CD45RO+ cells when compared with other. Phosphorylation levels of T-bet are significantly high in CD8+CD45RO+ cells and increased in CD8+CD45RA+ as well as CD4+CD45RA+ cells when compared with CD4+CD45RO+ cells. Western blot analysis results are displayed in Figure 1.

In general enhanced phosphorylation of p38 was observed in CD4+CD45RO+ cells when compared with CD4+CD45RA+ cells. When cells were incubated with p38 MAP kinase (SB203580) inhibitor prior to stimulation, a significantly decreased phosphorylation of p38 MAPK ($p < 0.05$) was observed (data not shown). p38 MAP kinase (SB203580) inhibitor inhibited STAT6 phosphorylation and the data clearly indicates a putative role for p38 in the induction of STAT6 since p38 inhibitor significantly down-modulated STAT6 phosphorylation [18]. In some experiments when anti-IL-4 antibody (10 ug/ml) was included in the culture, inhibition of STAT6 phosphorylation was observed (data not shown).

Stimulation with CD3+CD27XL was used as positive control. As shown in Table 3, CD4+CD45RA+ cells shifted towards Th1 cytokine phenotype, and CD4+CD45RO+ cells shifted towards Th2 phenotype. Both CD+CD45RA+ and CD8+CD45RO+ exhibited Th1 profile as shown in Table 3.

It is also clear from these studies that P38 MAPK, STAT6, and GATA3 appear to be associated with Th2 responses and MAP kinase ERK2, STAT 1, and T-bet appear to be associated with Th1 responses. Association of CD27XL in both CD8+CD45RO+ cells as well as CD8+CD45RA+ cells resulted in T-bet activation and IFN-γ secretion. TNF-alpha production was enhanced predominantly in the CD4+45RA+ T cells compared with the CD4+CD45RO+ T cells and IL-4 production was enhanced in CD45RO+ T cells. Phosphorylation of STAT1 and STAT6, T-bet and GATA3 was seen in 24 hours. Cytokine secretion was seen in 48 to 72 hours. STAT1 activation was associated with T-bet induction. The differentiation of the CD4+ T cell towards Thl or Th2 is regulated by the transcription factors T-bet and GATA3, respectively. IL-4 is the signature cytokine for Th2 cells whereas INF gamma is the signature cytokine for Thl cells. It has been shown that STAT6 is expressed in

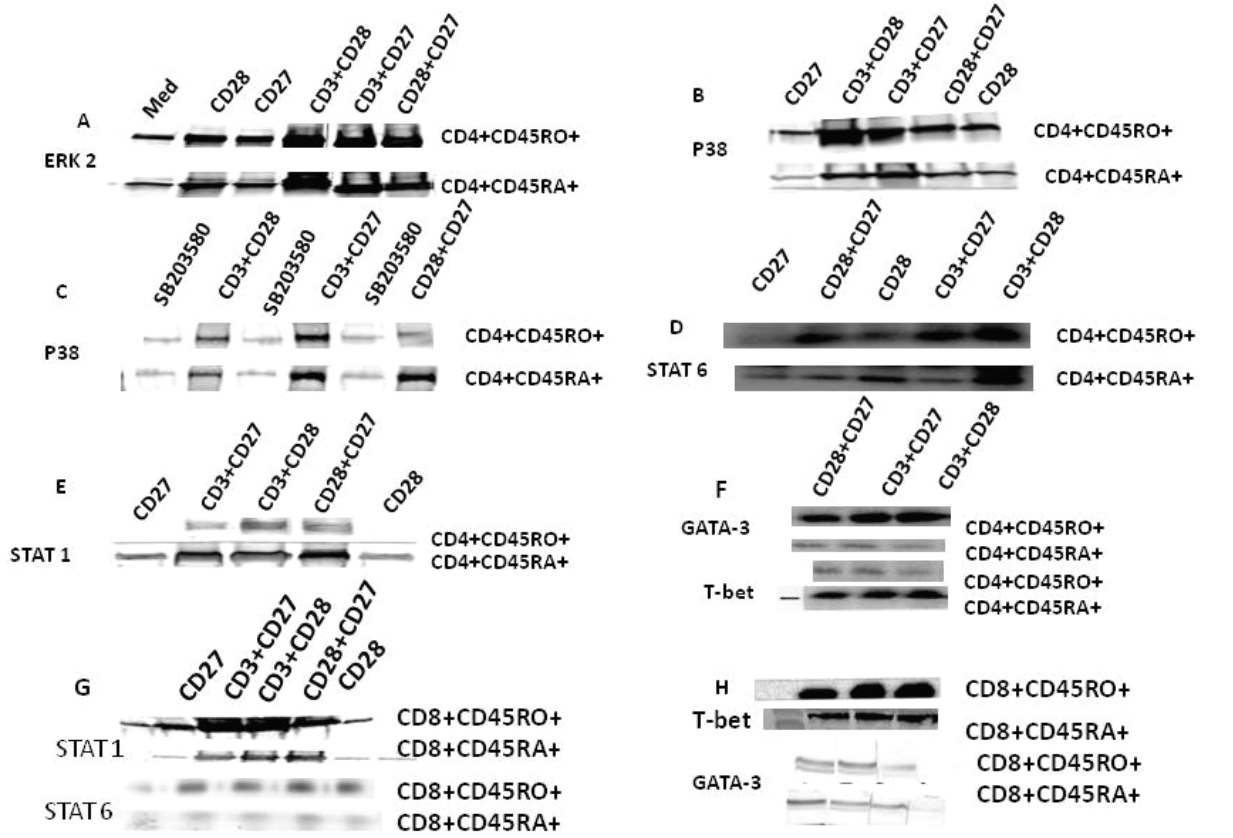

Figure 1. Western blotting, ERK2, p38, STAT6, STAT1, GATA3, and T-bet. Cell purification, cell stimulation, cell lysis, immunoprecipitation and Western Blotting were performed as described in 'Materials and Methods'. Figure 1A represents phosphorylation of ERK2 in CD4+CD45RO+ and CD45RA+ T cells. Figure 1B represents phosphorylation of P38 in CD4+CD45RO+ and CD4+CD45RA+ T cells and Figure 1C represents inhibitory effects of P38 inhibitor SB203580. Figure 1D and 1E represents phosphorylation of STAT6 and phosphorylation of STAT1 in CD4+CD45RO+ and CD4+CD45RA+ T cells, respectively. Figure 1F represents phosphorylation of GATA3 and T-bet in CD4+CD45RO+ and CD4+CD45RA+ T cells. Figure 1G represents phosphorylation of STAT1 and STAT6 in CD8+CD45RO+and CD8+CD45RA+ T cells. Figure 1H represents phosphorylation of T-bet and GATA3 in CD8+CD45RO+ and CD8+CD45RA+ T cells. Data is a representation of three independent experiments.

B cells and T cells upon stimulation with IL-4 and STAT6 is a central mediator of IL-4-induced gene responses and STAT4 is a central mediator of INF gamma-induced gene responses.

DISCUSSION

The TNF-R superfamily of proteins gained considerable attention in the last decade with a surge in development of immunomodulatory biologics to treat cancer, infectious diseases and autoimmunity [1, 19, 20]. The CD27-CD70 pathway appears to be important in cell survival, maintenance of memory cell function, anti-tumor cellular immunity and autoimmunity [3-7]. Its expression increases upon T cell activation, and is lost at the fully differentiated effector phase [2]. Our studies indicate positive loops between STAT6, GATA3 and IL-4 and STAT1, T-bet and IFN-γ in CD45RA-positive and RO-positive T cells. Th1 cells mediate cellular immunity by production of IFN-γ, which is critical for eradication of intracellular pathogens. In contrast, Th2 cells produce IL-4, IL-5, and IL-13, which in combination induce humoral immunity, allergic inflammation, and promote host resistance, particularly to intestinal nematodes [15, 16]. It has been suggested that the subset of T cells with low CD27 expression has high capacity to produce IFN-gamma [21]. It implies that low CD27

Table 2. Intensities of bands.

Protein	CD4+CD45RA+		CD4+CD45RO+		CD8+CD45RA+		CD8+CD45RO+	
	CD3+ CD27XL	CD28+ CD27XL	CD3+ CD27XL	CD28+ CD27XL	CD3+ CD27XL	CD28+ CD27XL	CD3+ CD27XL	CD28+ CD27XL
ERK	1700 ± 80	1510 ± 70	1480 ± 80	1250 ± 45	1650 ± 120	1710 ± 110	1660 ± 100	1700 ± 150
p38	1200 ± 50	1000 ± 65	1750 ± 40	1450 ± 110	950 ± 90	600 ± 80	900 ± 100	1100 ± 80
STAT6	700 ± 65	930 ± 50	1400 ± 100	1150 ± 90	500 ± 50	330 ± 50	650 ± 70	400 ± 55
STAT1	1300 ± 80	1350 ± 60	600 ± 30	700 ± 50	900 ± 45	1100 ± 90	1600 ± 110	1230 ± 60
GATA3	500 ± 60	400 ± 40	1800 ± 140	1420 ± 100	500 ± 45	400 ± 30	600 ± 80	400 ± 90
T-bet	1400 ±130	1300 ± 110	500 ± 50	600 ± 90	1500 ± 110	1400 ± 100	1800 ± 140	1700 ± 160

Table 3. Cytokine concentrations in pg/ml and proliferation.

Response	CD4+CD45RA+		CD4+CD45RO+		CD8+CD45RA+		CD8+CD45RO+	
	CD3+ CD27XL	CD28+ CD27XL	CD3+ CD27XL	CD28+ CD27XL	CD3+ CD27XL	CD28+ CD27XL	CD3+ CD27XL	CD28+ CD27XL
IL-4	800 ± 60	600 ± 50	1400 ± 60	1000 ± 50	400 ± 60	200 ± 50	400 ± 65	300 ± 50
IFN-gamma	1200 ± 90	800 ± 50	1100 ± 60	800 ± 50	1560 ± 90	1200 ± 50	1600 ± 90	1200 ± 150
TNF-alpha	1100 ± 60	700 ± 50	650 ± 60	500 ± 50	1400 ± 60	1000 ± 80	1250 ± 110	1200 ± 50
Proliferation	21,000 ± 690	14,000 ± 390	18,000 ± 550	16,000 ± 800	18,500 ± 690	14,000 ± 400	18,000 ± 450	16,000 ± 600

expression is characteristic of fully differentiated effector CD4+ T lymphocytes [21]. This may explain the increased T-bet phosphorylation in CD45RA+T cells.

T-bet plays a critical role in controlling IFN-γ expression, Th1 polarization, and CD8+ cytotoxic T cell function (4-5). Analysis of T-bet-deficient mice has shown that CD4 T cells lacking T-bet are severely impaired in their ability to produce IFN-γ, susceptible to Leishmania major infection, and have a marked *in vivo* shift of the Th1/Th2 balance toward the Th2 pathway [20-23]. T-bet was initially suggested to be induced by IL-12/STAT4 signals, but later investigation demonstrated that it is regulated by IFN-γ/STAT1. In addition to being positively regulated, T-bet has been shown to be negatively regulated by TGFβ, the Th2 transcription factor GATA3, and the Tec family tyrosine kinase Itk [20-23].

Zhu *et al*. [24] found that STAT6 is necessary for IL-4 induced functions in CD4+ T cells, and that STAT6 is not only necessary but also sufficient for the IL-4 effects in Th2 differentiation and cell expansion. Since CD28 along with CD27 was sufficient to provide the signals required for Ag-independent Th2 cell differentiation, it offers a cellular mechanism to generate Th2 effector cells. Either GATA3 expression down-regulates T-bet expression or T-bet expression down-regulates the expression of GATA3. T-bet is amplified by STATl signals generated in response to IFN-γ. T-bet plays essential roles in several facets of type 1-related, inflammatory T cell differentiation. We previously reported that phosphorylation of p38 is important in regulation of STAT6 as well as IL-4 synthesis; STAT6 regulates the expression of IL-4 inducible gene GATA3 and GATA3 expression is dependent on STAT6 signaling [17, 18].

Hatwig UF *et al*. suggested that the ability of IL-4 to prevent CD27L expression may limit CD27-CD27L interaction to Th1-type T cell responses [25]. Within the intracellular domains of human IL-4R alpha, STAT6 interacts with the second, third, and fourth conserved tyrosines (Y575, Y603, Y631) [26, 12]. STAT6 is recruited to the IL-4R complex by binding to any of three phosphotyrosines in IL-4R alpha. It becomes tyrosyl phosphorylated at its C-terminus through the action of Janus kinases 1 and or 3. Phosphorylated STAT6 dimerizes, migrates to the nucleus, binds to specific DNA elements, and, together with other transcription factors, activates transcription of some IL-4-induced genes [26].

Schmitter D *et al*. [7] concluded that CD27+CD70 represents another co-stimulatory pathway involved in T-cell-mediated immune responses to follicular lymphoma cells. These studies suggest that some of these co-stimulatory pathways should be taken in to consideration in the enhanced CTL activity and adaptive immunotherapy. In this context, Schmitter D *et al*. [27] investigated the effect of anti-CD70 mAb on experimental autoimmune encephalomyelitis (EAE), and their data indicate that the CD70-CD27 interaction plays a pivotal role in the development of cell-mediated autoimmune disease.

Thus activation of human CD27 (TNFRSF7) with a mAb (Varlilumab) was demonstrated to result in T cell activation and anti-tumor activity in preclinical models, and is currently in early phase clinical trials in patients with advanced malignancies. They used an *in vitro* system using human peripheral blood T cells to characterize the varlilumab-mediated costimulatory effects in combination with TCR stimulation and assessed phenotypic, transcriptional and functionality changes [28, 29]. It should be further explored whether phenotype analysis of Th1 and Th2 subsets of T cells may offer insights into prognostic determinants for the evaluation of protection against viral infection or tumor promotion/ suppression.

CONCLUSION

Overexpression of CD70 by tumor cells has been shown to promote T cell dysfunction or apoptosis through CD27 signaling *in vitro* [30]. However, this effect was overridden by the immune activating properties of CD27 signaling when tested in tumor models. Regulatory T cells (Tregs) are known to express CD27, but the role of CD27 costimulation on these cells and their function is unknown. It is critically important to assess whether CD27 may play important role in tumor suppression and proliferation of Tregs [31]. The findings of De Miloto A *et al*. [32], suggest that plasma sCD27

may represent an alternative and simple marker to monitor immune activation during potent anti-retroviral HAART therapy.

ACKNOWLEDGEMENTS

We wish to express our gratitude to Dean's office, School of Health Professions and Nursing for supporting this work.

CONFLICT OF INTEREST STATEMENT

Neither author reports any conflicts of interest.

REFERENCES

1. Najajima, A., Oshima, H., Nohara, C., Morimoto, S., Yoshino, S., Kobata, T., Yagita, H. and Okumura, K. 2000, J. Neuroimmunol., 109, 188.
2. Hintzen, R. Q., de Jong, R., Lens, S. M., Brouwer, M., Baars, P. and van Lier, R. A. W. 1993, J. Immunol., 151, 2426.
3. Tesselaar, K., Xiao, Y., Arens, R., van Schijndel, G. M. W., Schuurhuis, D. H., Mebius, R. E., Borst, J. and van Lier, R. A. W. 2003, J. Immunol., 170, 33.
4. Rufer, N., Zippelius, A., Batard, P., Pittet, M., Kurth, I., Corthesy, P., Cerottini, J.-C., Leyvraz, S., Roosne, E., Nabholz, M. and Romero, P. 2003, Blood, 102, 1779.
5. Tomiyama, H., Matsuda, T. and Takiguchi, M. 2002, J. Immunol., 168, 5538..
6. Hendriks, J., Xiao, Y. and Borst, J. 2003, J. Exp. Med., 1369.
7. Denoeud, J. and Moser, M. 2011, J. Leuk. Biol., 89, 1.
8. Naguno, H., Agemetsu, K., Shinozaki, K., Hokibara, S., Ito, S., Takamoto, M., Nikaido, T., Yasui, K., Uehara, Y., Yachie, A. and Komiyama, A. 1998, J. Immunol., 161, 6496.
9. Shahinian, A., Pfeffer, K., Lee, K. P., Kundig, T. M., Kishihara, K., Wakeham, A., Kawai, K., Ohashi, P. S., Thompson, C. B. and Mak, T. W. 1993, Science, 261, 609.
10. Quelle, F. W., Shimoda, K., Thierfelder, W., Fischer, C., Kim, A., Ruben, S. M., Cleveland, J. L., Pierce, J. H., Keegan, A. D. and Nelms, K. 1995, Mol. Cell Biol., 15, 3336.
11. Pai, S-Y., Truitt, M. L., Ting, C. N., Leiden, J. M. and Glimcher, L. H. 2003, Immunity, 19, 863.
12. Lantelme, E., Mantovani, S., Palermo, B., Campanelli, R., Sallusto, F. and Gachino, C. 2001, Immunology, 102, 123.
13. Stanford, I. and Peng, L. 2006, Cellular and Molecular Immunology, 3, 87.
14. Peng, S. L. 2006, Cell. Mol. Immunol., 3, 87.
15. Sullivan, B. M., Jobe, O., Lazarevic, V., Vasquez, K., Bronson, R., Glimcher, L. H. and Kramnik, I. 2005, J. Immunol., 175, 4593
16. Ravindran, R., Foley, J., Stoklasek, T., Glimcher, L. H. and McSorley, S. J. 2005, J. Immunol., 175, 4603.
17. Tamma, S. M., Balan, S. P. and Chung, K. W. 2006, J. Leuko Biol., 79, 876.
18. Tamma, S. M., Chung, K. W., Patel, T., Balan, S. and Pahwa, S. 2006, J. Leuko Biol., 79, 1339.
19. Hintzen, R. Q., Lens, S. M., Beckmann, M. P., Goodwin, R. G., Lynch, D. and van Lier, R. A. 1994, J. Immunol., 152, 1762.
20. Neurath, M. F., Weigmann, B., Finotto, S., Glickman, J., Nieuwenhuis, E., Lijima, H., Mizoguchi, A., Mizoguchi, E., Mudter, J., Galle, P. R., Bhan, A., Autschbach, F., Sullivan, B. M., Szabo, S. J., Glimcher, L. H. and Blumberg, R. S. 2002, J. Exp. Med., 195, 1129.
21. Lucas, S., Ghilardi, N., Li, J. and de Sauvage, F. J. 2003, Proc. Natl. Acad. Sci. USA, 100, 15047.
22. Yang, Y., Ochando, J. C., Bromberg, J. S. and Ding, Y. 2007, Blood, 110, 2494.
23. Gollob, J. A., Murphy, E. A., Mahajan, S., Schnipper, C. P., Ritz, J. and Frank, D. A. 1998, Blood, 91, 1341.
24. Zhu, J., Quo, L., Watson, C. J., Hu-Li, J. and Paul, W. E. 2001, J. Immunol., 166, 7276.
25. Hartwig, U. F., Robbers, M., Wickenhauser, C. and Huber, C. 2002, Blood, 99, 3041.
26. Maneechotesuwan, K., Yao, X., Ito, K., Jazrawi, E., Lee, K., Usmani, O., Barnes, P. and Adcock, I. 2007, J. Immunol., 178, 2491.
27. Schmitter, D., Bolliger, U., Hallek, M. and Pichert, G. 1999, Br. J. Hemaetology, 106, 64.

28. Ramakrishna, V., Sundarapandiyan, K., Zhao, B., Bylesjo, M., Marsh, H. C. and Keler, T. 2015, J. Immunother Cancer, 3, 37.
29. Burris, H. A., Infante, J. R., Ansell, S. M., Nemunaitis, J. J., Weiss, G. R., Villalobos, V. M., Sikic, B. I., Taylor, M. H., Northfelt, D. W., Carson, W. E. 3rd., Hawthorne, T. R., Davis, T. A., Yellin, M. J., Keler, T. and Bullock, T. 2017, J. Clin. Oncol., 35, 2028.
30. Sakanishi, T. and Yagita, H. 2010, Biochem. Biophys. Res. Commun., 393, 829.
31. Claus, C., Reither, C., Schurch, C., Matter, M. S., Hilmenyuk, T. and Ochsenbein, A. F. 2012, Cancer Res., 72, 3364.
32. De Milito, A., Aleman, S., Marenz, R., Sonnenborg, A., Zazzi, M. and Chiodi, F. 2002, Clin. Exp. Immunol., 127, 486.