Role of CD4 Th1,Th9,Th17,Th22 cells in bladder cancer

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Abstract

Tumor infiltrating lymphocytes (TILs) are candidate as crucial biomarkers, since certain cancers have high lymphocyte infiltration that obtain significant role in specific cancer types that can be contributed in tumor initiation, pathogenesis, progression and may give aid in disease prognosis and therapy. CD4 T cells found to have an important role in bladder cancer immunopathogenesis but still there is no clear view with an argument about their role. In this study we assess the CD4 Th1,Th9,Th17,Th22 cellsinfiltration in tumor and autologous normal microenvironments of urinary bladder and subsequently their significance in antitumor immunology with their clinicopathological influences of urinary bladder cancer.

Keywords: Th1,Th9,Th17, Th22, CD4, subsets, bladder, cancer

Introduction

The CD4 T cells may comprise a resident immune cells population in healthy human bladder (Christmas *et al*., 1994). When they interact with reactive dendritic cells it would significantly be attracted to site of immune reaction (Ahrends *et al*., 2018). As The reactive DC interact with the naïve CD4 T cells at their associated TCR ligand they would develop different phenotypes this occurs in presence of certain cytokine background, as in the existence of IL-12 with or without IFN-α the naïve T cell would acquire Th1 phenotype (Biedermann *et al*., 2004).

After polarization Th1 cells would migrate to tumor microenvironment to secret IFN-γ and enhances the cell mediated cytotoxicity exerted by macrophage and CD8 T cells. An experimental study on bladder cancer of murine bladder tumor and lymph nodes cellular immune infiltrate observed a CD4 T cells expansion which secrete IFN-γ at same time, the IFN-γ neutralization it would inhibit the immune responses against tumor exerted by immune checkpoint blocking proposing the crucial role of the Th1 cells (Sato *et al*., 2018).

Advanced stages of bladder cancer have been associated with augmented methylation so the IFN-γ loci would be decreased (Ahlén-Bergman *et al.*, 2018). A considerable hypomethylation at all functional type loci have been found in patients who express a full response to chemotherapy, merely most obviously at the IFN-γ locus proving the Th1 cells antitumor responses (Ahlén Bergman *et al.*, 2018).

Naïve $CD4^+$ T cell differentiates under the effects of both IL-4 and TGF-β to Th-9 while it can also be differentiated from the $CD4^+$ Th2 cell in the presence of TGF-β solely, so it is a separate $CD4^+$ cells and not a Th2 cells subtype. The Th9 cells activity have been linked with anti-helminthic immune activity and many autoimmune diseases (Raphael*et al*., 2015; Li *et al*., 2017). It secrets of IL-10 and IL-9 induces an inflammatory processes via IL-9 gene expression regulation (Schmitt *et al*., 2014).

The Th9 cells have a higher antitumor influence than Th1 subset (Lu *et al.*, 2018). Their antitumor effects mediated by both innate and adaptive immune response figure (2-6), IL-9 have been found to prolong DC survival and at the same time, stimulate mast cells activities (Purwar*et al*., 2012). In addition to that IL-9 found to enhance the DC and $CD8^+$ T cells recruitment

to tumor bed (Lu *et al.*, 2012). Also, Th9 cells found to enhance the cytotoxic abilities of both $CD8^+$ T cell and NK cell through the secretion of IL-21 which in turn enhances the production of IFN-γ (Vegran*et al*., 2014).

The Th9 cells antitumor strategy did not involve adaptive immunity, since it has been found to be applied through IL-9 activation of mast cell (Abdul-Wahid *et al*., 2016). There is a controversial opinion about Th9-derived IL-9 in tumor immunology, since Hoelzinger*et al.*, proposed that IL-9 neutralization ameliorated tumor burdens (Hoelzinger*et al*., 2014). In addition to that there is an assumption about IL-9 role in enhancing immune tolerance via stimulating Tregs functions and inhibit the development of immunological memory and subsequently suppress tumor specific immune responses (Elyaman*et al*., 2009; Hoelzinger*et al*., 2014).

Whereas another study proposed that IL-9 had an immune defense mechanism and found to fight melanoma cells via multiple effector mechanisms (Lu*et al*., 2012). In a ($ROR^{-/-}\gamma$)-deficient mice the induced melanoma growth has been inhibited, characterized by high infiltration of tumor microenvironment with $CD4^+$ and $CD8^+$ T cells with high IL-9 secretion. When IL-9 neutralized these effects completely reversed, signifying an antitumor immune response (Glimelius*et al*., 2006; Feng *et al*., 2011; Lu *et al*., 2012).

In an experimental mice which induced to over express IL-9 resulted in developing thymic lymphomas, this indicating T cell malignancy can be developed from IL-9 high expression (Renauld*et al.*, 1994). IL-9 have been identified to be a T cell growth factor (Uyttenhove*et al*., 1988). many hematological human tumors have been found to be developed as a result of IL-9 over expression like B cell lymphoma (Fischer*et al*., 2003).

Effects of Th-17 cells in bladder cancer still not clear, but Immune responses to BCG appeared to be IL-17 dependent production, (Yuasa*et al*., 2009). On the other hand, IL-17 can act as important factor for induction of angiogenesis so act as pro-tumorigenic in the absence of other therapies (Wang*et al*., 2009). The IL-17 act as a vital mediator in the pathogenesis of autoimmune disease, the high peripheral blood with Th-17 cells, associated with disease activity, Th-17 cells have an important influence in immune defenses in case of microbial infections and seems to have vital role in the disease mechanisms of autoimmune disorders (Shah *et al*., 2010). There is a contradiction about the role of Th17 cells in antitumor immune responses (Kryczek*et al*., 2007; Sfanos*et al*., 2008).

However, Th17 expresses certain chemokine receptors selectively from certain origins (Acosta-Rodriguez *et al*., 2007; Liu *et al*., 2008). The high expression of CCR4 and CCR6 by tumor-infiltrating Th17 cells, may reflect their relation with the migration and sustainability of intra-tumoral Th17 cell (Chi *et al*., 2010).

The Th22 cells can be identified as a new $CD4^+$ T-cell subset which secrete IL-22, and do not secrete IL-17 IFN-γ (Eyerich*et al*., 2009; Tarifari*et al*., 2009). These cells express (CCR6) and CCR4 which are express by Th-17 cells as well also Th22 cells expressing CCR10 at the same time do not express CD161 which is expressed by Th-17 (Duhen*et al*., 2009). Transcription AHR is the key factor of Th22 cells and expresses low level of T-bet and RORC (Veldhoen*et al*., 2008). The Th22 differentiated from the naive $CD4^+$ T-cell under the effects of both IL-6 and (TNF-α)(Tarifari*et al*., 2009).

The IL-22 is the main effector cytokine of Th22 cell in addition IL-26, and IL-33. The IL-22 perform its biological effects via a receptor complex comprising of IL-10R2 and IL-22R1 (Plank*et al*., 2017). The targets of IL-22 are Th22 cells and other immune cells like (natural killer (NK), Th1, Th17, and NKT cells) of tissues at the outer body barriers, like the epithelial cells in the respiratory systems, renal system, and digestive system. IL-22 functioning by enhancing the microbial defenses against the microbial invasion, and re-arranging non-immune cells of the tissues, protects the tissues from being damage, so it regulates tissue protection and homeostasis (Bleicher *et al*., 2008; Wolk*et al.,* 2010).

Both Th22 cells and IL-22 can be contributed in tumor pathogenesis likegastric cancer (Zhuang*et al*., 2012), multiple myeloma (Di Lullo *et al*., 2015), colorectal cancer (Huang*et al*., 2015), hepatocellular carcinoma (Qin *et al*., 2014). In gastric andhepatocellular carcinoma Th22 cells found to be associated with tumor progression (Qin *et al*., 2014). Interleukin-22 stimulate the secretion and release of diverse factors that enhance tumor progression in pancreatic cancer cell lines, like IL-10, TGF-β, and VEGFα. Pancreatic ductal adenocarcinomas found to expresses high level of IL-22R1 and IL-22, at the same time associated with lymph node invasion (Curd*et al*., 2012 Wen et al., 2014). High level of the circulating Th22 in patients with Epithelial Ovarian cancer, and it was correlated positively with tumor progression (Wang *et al.,* 2017).

Higher percentage of Th22 cells have been found in advanced clear cell carcinoma patients, exploring a vital effect of Th22 in clear cell carcinoma growth and progression (Zhang*et al*., 2015). However, at least to my knowledge the role of infiltrating Th22 in urinary bladder cancer not been assessed yet.

3.Materials and Methods

3.1. Patients and samples:

Autologous tissues (normal and tumor) samples were taken from 17 patients undergoing tumor resection by Trans Urethral Resection of Bladder Tumor surgery (TURBT) for primary urinary bladder cancer at the Basra Teaching Hospital after written informed consent. Follow up period for assessment of disease recurrence was 1 years. 17 autologous (tumor and normal) Fresh samples were directly transferred into Dulbecco's Minimal Essential Medium (DMEM) media EuroClone, S.P.A Italy, catalogue number ECM0728L, then tissue specimen placed on ice tissue samples were transferred immediately to Al Bayan group for advanced lab. Diagnostic, to assess the $CD4^+$ cellular infiltration in bladder cancer patients by the mean of flow cytometry, for process of single cell sample generation and flow cytometric analysis.

3.1.1. Inclusion Criteria:

- Patients who diagnosed with urinary bladder cancer
- Primary tumor

3.1.2. Exclusion Criteria:

- Secondary tumors
- Recurrent disease
- Inflammatory disease like infection
- Patients on immune suppressive or modulating therapy.
- Patients known case of autoimmune disorders.

3.2.CD4 T- Cell subset assessment by flow cytometry

3.2.1. Single cell formation process for assessment by flowcytometry:

Preparing 2X TTDR To make 2X TTDR ((Tumor-Tissue Dissociation Reagent) from BD Bioscience USA), catalogue number (661563):

- Five mL of DMEM added to the amber vial containing TTDR.
- Gently agitated periodically for 15 minutes at room temperature to ensure complete reconstitution of the dried reagent.
- The reconstituted TTDR transferred to a labeled 50-mL conical centrifuge tube.
- Discarded the amber vial.
- Then Stored at 4°C until needed.

After weighed the tissue specimen, then to minced the tissue placed into a fresh, labeled 100 × 20-mm glass petri dish containing 5 mL of 37°C DMEM. Two scalpels were used to mince the tissue to minute pieces in the petri dish figure (3-1). The resulting tissue pieces was as small as possible Approximately 30 minutes before tissue mincing has been completed, placed the tube containing 2X TTDR (Tumor-Tissue Dissociation Reagent) from BD Biosciences (USA) in 37°C water bath not longer than 30 minutes prior to use. The contents of the petri dish (DMEM and tumor pieces) transferred into conical tube containing 37°C 2X TTDR. The final volume in the conical tube should be 10 mL (5 mL warm TTDR +5 mL minced tumor in DMEM).

3.2.2. Digesting the minced tissue:

The tubes containing the minced tissue and TTDR were incubated at 37°C for 30 minutes with mild but frequent agitation. After incubation, 25 mL of 1% BSA/DPBS/2 mM EDTA was added to the conical centrifuge tubes containing the dissociated tissue to bring total volume to 35 ml. Passed the contents of each tube through a fresh 70-µm cell strainerinto a fresh labeled conical tube. After washing the strainer with 10 ml DMEM media Then after the tubes Centrifuged at 250g for 8 minutes at room temperature.

The supernatant removed then after resuspended the pellets in 2 mL of 1X BD Pharm Lyse™ solution from BD Bioscience, USA. Incubated for 15 minutes at room temperature. Then 40 mL 1% BSA/DPBS/2 mM EDTA added and Centrifuge at 250g for 8 minutes at room temperature. Then removed the supernatant and resuspend pellets in 2 mL DPBS/2 mM EDTA.

3.2.3. Cell stimulation for intracellular staining

Leukocyte Activation Cocktail with Golgi Plug from BD Bioscience,

(USA), catalogue number (550583), used for T-cell activation to secrete cytokines for assessment by intracellular immunofluorescent staining.

- The cocktail was thawed at 37°C in a water bath.
- Two µL of the cocktail added for every 1 mL of the dissociated samples and mixed thoroughly.
- Then after Placed in a 37°C humidified CO2 incubator for 6 hrs.
- After activation the cells washed with FACS Staining Buffer to be used in flow cytometric assessment.

3.2.4. Fluorochrome-conjugated monoclonal antibodies staining

- The cells washed with wash buffer
- Permeabilization Buffer added then washed before the final wash step
- Resuspended the cells in staining buffer,
- Then 50 µl of the suspension added to each tube.
- Fluorochrome-conjugated monoclonal antibodies added (5 µl)
- The mixture incubated in the dark for 15-20 min at 4°C.
- The gating strategy planned by BD, Bioscience instructor.
  - Th1 cell ($CD3^+$ $CD4^+$INF-γ^+ IL-17^+)
  - Th22 cell ($CD3^+CD4^+$ IL-22^+IL-17^-)
  - Th9 cell ($CD3^+CD4^+CD25^-CD127^+$ $CD45RA^+CCR4^-$ $CCR6^+$)

3.3.Statistical analysis

IBN SPSS statistics version 22 program for statistical analysis have been used. The data expressed as M ±SD. Wilcoxon Signed Ranks Test used for paired samples, while Kruskal Wallis Test to assess multiple types of samples. Spearman's Correlations test used to assess the presence of a correlation between the assessed parameters in autologous tumor and normal microenvironments of urinary bladder cancer patients. The statistically significant difference considered at $P<0.05$.

4. Results

The mean percentage of Th1 cell infiltration in urinary bladder tumor microenvironment (47.1±16.0) higher than their infiltration in normal tissue (45.1±11.7), but this difference was not significant (p value > 0.05) table (1). We found no correlation between Th1 cell infiltration in urinary bladder

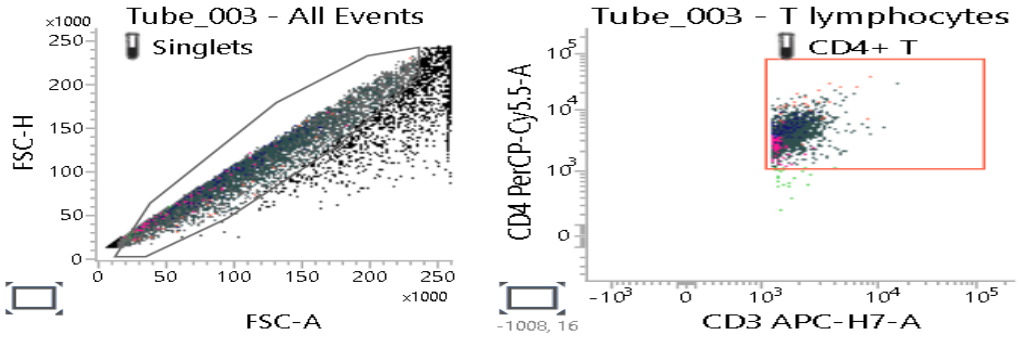
tumor microenvironment and disease stage, tumor grade, tumor size or LN involvement table (2). There is significant negative correlation between Th1 cell and both Th9 (p value ≤ 0.01) and Th22 cell infiltration in tumor microenvironment (p value ≤0.05) table (3).

The Th9 cell infiltration assessment results in normal urinary bladder tissue (55.2±36.6) was higher than their infiltration in tumor tissue (42.6±41.3), however this difference was statistically not significant (p value > 0.05) table (1). There was no correlation between Th9 cell infiltration in tumor microenvironment and disease stages, tumor grade, tumor size or LN involvement table (2).

The mean percentage of Th17 cell infiltration in tumor microenvironment (2.20±1.72) higher than mean percentage of Th17 cell infiltration in normal microenvironment (1.32±1.3) but this was not significant difference (p value > 0.05) table (1). There was significant negative correlation between Th17 cell infiltration in tumor microenvironmentand bladder cancer stage (p value≤ 0.05), at the same time there was no correlation between Th17 cell infiltration in tumor microenvironment and tumor grade, LN involvement or tumor size (p value > 0.05) table (2).

The Th22 cell mean percentage of infiltration in normal tissue (26.5±8.0) was significantly higher than their infiltration in tumor tissue (20.6±10.2), (p value < 0.05) table (1). There is no significant correlation found between Th22 cell infiltration in tumor microenvironment and disease stage, tumor grade, LN involvement or tumor size (p value >0.05) table (2), there is significant positive correlation between Th22 and Th9 cells infiltration in tumor tissue table (3).

a-



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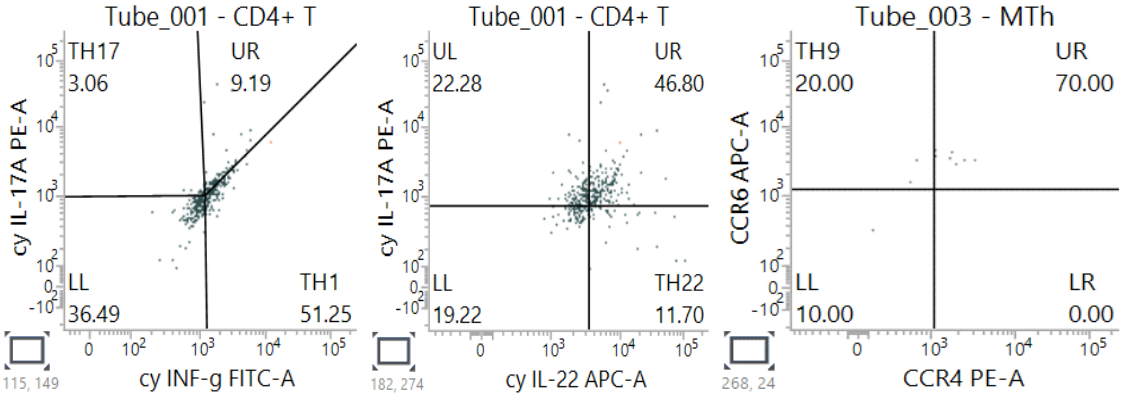

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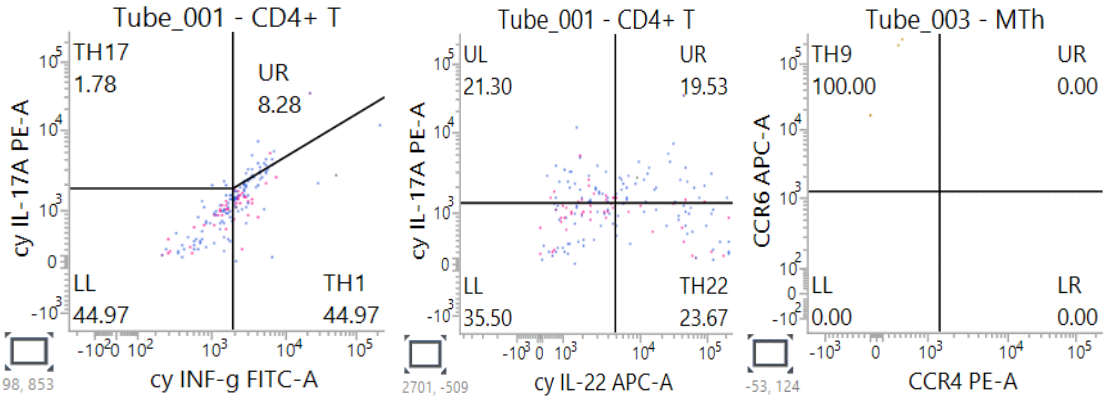

Figure (1): a-CD4 T lymphocytes b-assessment of Th1, Th9,Th17, Th22 cell infiltration in tumor microenvironment of urinary bladder cancer by flowcytometry.c-assessment of Th1, Th9, Th17 Th22 cell infiltration in normal urinary bladder microenvironment by flowcytometry.

Table (1): The comparison of CD4 T-cell subsets infiltration between tumor and normal urinary bladder cancer tissue.

CD4 T-cell subset	Mean±SD	Min.-Max.	P value
Th1 normal	45.1±11.7	27.8-61.4	0.59
Th1 tumor	47.1±16.0	25.9-75.1	
Th9 normal	55.2±36.6	0-100	0.57
Th9 tumor	42.6±41.3	0-100	
Th17 normal	1.32±1.3	0.26-4.7	0.177
Th17 tumor	2.20±1.72	0.1-4.6	
Th22 normal	26.5±8.0	13.4-37.8	0.041*
Th22 tumor	20.6±10.2	10.8-40.4	

*significant difference (p value < 0.05)

Table (2):CD4 T-cell subset prevalence in tumor microenvironment in correlation to bladder cancer patient disease stage, tumor size, LN involvement.

Spearman's correlation test		Stage	Tumor grade	Tumor size	L.N.
Th1	R.	0.261	0.087	0.416	0.46
	P value	0.439	0.799	0.204	0.15
Th9	R.	-0.30	-0.049	-0.46	-0.34
	P value	0.369	0.887	0.146	0.30
Th17	R.	-0.611	-0.084	-0.070	-0.36
	P value	0.046*	0.806	0.839	0.27
Th22	R.	-0.154	0.026	-0.53	-0.30
	P value	0.651	0.941	0.088	0.35

- R. (Ratio),
- *Significance (p value ≤ 0.05)

Table (3): CD4 T-cell subsets with positive or negative correlation of infiltration in tumor microenvironment.

Spearman's correlation test	R.	Sig.
Th22-Th1	-0.873**	0.000
Th1-Th9	-0.654*	0.029
Th9-Th22	0.687*	0.019

- Significant at the (* 0.05, **0.01) level
- R. (Ratio),Sig.(significance).

4.Discussion

There is a critical association between T cell immune responses and tumor progress (Zhang *et al*., 2003;Dieu-Nosjean*et al*., 2008; Hu *et al*., 2017). Th1 cell signified by their important role in enhancing antitumor cell mediated cytotoxicity exerted by macrophages and CD8 T-cells (Joseph and Enting, 2019), in experimental murine bladder cancer Th1 cells found to be expanded, at the same time neutralization of IFN-γwhich is secreted by Th1 cells result in inhibition of anti-tumor immune responses exerted by immune check points inhibitors (Sato *et al*., 2018). While another study found IFN-γ enhance tumor cells to express PD-L1 and consequently leading to immune therapy resistance so this reflecting IFN-γ role as a tumor enhancer (Abiko*et al*., 2015).

Many types of cancer exhibited high Th1 cell infiltration in tumor microenvironment and it was associated with good prognosis (Zhang *et al*., 2003; Galon *et al*., 2006; Sharma *et al*., 2007; Fridman *et al*., 2012). This study found that Th1 cell infiltration in urinary bladder cancer tumor microenvironment was higher than their infiltration in normal tissue, at the same time found no correlation between Th1 cell tumor infiltration with disease stage, grade, tumor size or lymph node involvement. These findings consistent with study on cervical cancer, but they found a significant higher prevalence of Th1 in patients with lymphatic involvement (Zhang *et al*., 2015). High expression of Th1 genes found in patients with chronic lymphocytic leukemia but this found to be not associated with late disease stages (Palma *et al*., 2017). Another study about urinary bladder cancer patient response to BCG treatment they found significantly lower Th1 cell peritumoral infiltration in patient who respond to BCG therapy than the non-responder, at the same time lower Th1 cell peritumoral infiltration associated with prolong recurrence free survival (Martínez *et al*., 2019). All the above

gives a clue about a possible pathological role of Th1 cell in urinary bladder cancer.

Th9 cells found to enhance anti-tumor immune defenses in solid tumors through both innate and adaptive immune defenses (Zheng and Lu, 2020). In the same context higher level of Th9 cells in peripheral blood in melanoma patients who respond to immune check point therapy than the non-responder (Nonomura*et al.*, 2016). Studies on lung cancer and hepatocellular carcinoma have found higher Th9 cell infiltration in tumor tissue than non-tumor tissue and it was associated with poor survival as well (Salazar*et al.*, 2020; Tan *et al*., 2017).

In an experimental study on different Th cell subsets to assess their anti-tumor cytolytic activity they have found that Th9 cell have the higher robust cytolytic effect on cancer cells (Chen*et al*., 2020). Also, Th9 cell have a hyperproliferative effects through NF-κB pathway activation and they tend to survive for longer time (Miao*et al.*, 2017).

In the present study we found higher Th9 cell infiltration in normal tissue than their infiltration in tumor tissue and it was statistically not significant probably due to small sample size, while in study on muscle invasive urinary bladder cancer they have found higher infiltration of IL-9^+cell in tumor microenvironment associated with impaired cell mediated cytotoxicity and higher T-reg infiltration, at the same time they have found significant correlation with disease grade and no significant differences in correlation to disease stages or lymphatic involvement (Zhou *et al*., 2020), since IL-9^+ also produced by cells other than Th9 cells like Tc9 cells, Vδ2 T cells, NKT cells and mast cells, so this can't reflects the exact role of Th9 cell (Wan *et al*., 2020). At the same time, no correlation was found between Th9 cell and tumor stage, grade, size or lymph node involvement. From all above suggesting an immune suppressed tumor microenvironment of bladder

cancer since it has a lower infiltration with Th9 cells and Th9 cell can be an important therapeutic strategy in urinary bladder but still it needs more studies to establish their role in urinary bladder cancer.

There is a contradictory about Th17 cell role in tumor immunology (Kryczek*et al*., 2007; Sfanos*et al*., 2008). In melanoma mice model Th17 cells found to destroy melanoma tumors directly and even stronger than Th1 cells (Martin-Orozco *et al*., 2009). Conversely, high infiltration of Th17 cell have found in tumor microenvironment of pancreatic and colonic cancer, and it was correlated with poor disease prognosis (Lanca*et al*., 2012; Dai*et al*., 2014). Tumor infiltrating Th17 cell in bladder cancer found to be highly expressing CCR4 and CCR6 which are a homing receptor responsible for Th17 cells migration to tumor site and subsequently their retention with in the tumor (Chi *et al*., 2010).

This study found higher Th17 cell prevalence in tumor tissue in comparison to normal tissue of urinary bladder, but this difference was not significant, also we find significant correlation between tumor stage and Th17 cell infiltration in tumor microenvironment, but no correlation found with grade, size or lymph node involvement and this possibly due to low sample size, our results consistent with Chi *et al*., findings who compare the Th17 cell prevalence in tumor microenvironment of bladder cancer patients with normal tissue healthy from healthy control (Chi *et al*., 2010). From all the above we can suggest a critical role of Th17 cell in tumor initiation and progression generally and particularly in urinary bladder cancer patients.

Th22 cell known as proinflammatory cell which play a critical role in autoimmune disease and other inflammatory disorders like ankylosing spondylitis and rheumatoid arthritis (Zhang *et al*., 2012). Th22 cells and IL-22 found to be contributed in tumor pathogenesis in many cancers type like

gastric cancer (Zhuang *et al*., 2012), multiple myeloma (Di Lullo *et al*., 2015), colorectal cancer (Huang *et al*., 2015),hepatocellular carcinoma (Qin *et al*., 2014).

Th22 cells level in peripheral blood have been found to be higher in patients with cervical cancerthan that for healthy control, also they found significant higher Th22 prevalence in patients with lymphatic involvement, while no significant difference found in relation to tumor stage or size (Zhang *et al*., 2015). The present study found a significantly lower Th22 cell infiltration in tumor tissue than the autologous non-tumor tissue and there was no correlation with tumor stage, grade, size, or lymph node involvement. Our finding consistent with a study on colorectal cancer patient which have found a significant lower circulating Th22 in comparison to the control group and it was negatively associated with disease stage and tumor progression (Ling *et al*., 2015). Liu *et al*. found a highly reduction of Th22 level in newly diagnosed patient with acute myelocytic leukemia in comparison to healthy control (Liu *et al*., 2012). Our finding suggesting a highly suppressed tumor microenvironment of urinary bladder cancer than normal tissue and this reduction in Th22 may associated at least partially and this make Th22 cell a possible therapeutic factor in treatment of urinary bladder cancer.

We observed a significant negative correlation between Th1 cell with Th9 and Th22 cells infiltration in tumor tissue, at the same time a significant positive correlation between Th9 cell and Th22 cell infiltration in tumor tissue of urinary bladder cancer. According to speculation of Abdelaziz et al., that Th9 cells differentiation would not occur directly from naïve $CD4^+$ T-cell, and it may pass through an intermediate form during which Th2 cell generated in order to reach the stable form represented by the Th9 (Abdelaziz*et al*., 2020).

A study for assessment non-muscle invasive bladder cancer patient response to intravesical BCG therapy that have found a significant lower Th1/total lymphocyte in peritumoral tissue of the responder than the non-responder, at the same time the BCG responder exhibited a significant higher Th2/Th1 ratio than the non-responder (Martínez *et al*., 2019).

The current study found a significant negative correlation between Th22 cell and Th1. Since lower Th1 prevalence and lower Th1/total lymphocyte found in non- muscle invasive bladder cancer patients who respond to BCG therapy, also associated with prolonged recurrence free survival (Martínez *et al*., 2019). From all the above and since this study found Th22 cell prevalence in tumor tissue lower than that in normal tissue we can speculate an important protective role of Th22 cell in urinary bladder cancer and their decrease in tumor microenvironment may associated with tumor initiation and promotion.

This study found a positive correlation between Th9 cell and Th22 cell prevalence in tumor tissue, at least to my knowledge there is no study assessed this type of correlation. Generally, there was an evidence about IL-9 role as a mediator for Th17 expansion (Nowak*et al*., 2009), at the same time Th22 cell expansion found to be associated with Th17 cell expression (Yu*et al*., 2014). Despite the controversial speculations and findings about the Th9 cell (Chen*et al*., 2020; Salazar*et al*., 2020), and Th22 cell role in tumor immunity (Huang *et al*., 2015; Ling *et al*., 2015), So we can suggest a possible relation between Th9 cell and Th22 cell expansion in urinary bladder tumor tissue which still need further assessment.

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