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Evaluation of Innate Lymphoid Cells (ILCs) Population in Mouse Model of Colorectal Cancer

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ABSTRACT

Background: Innate Lymphoid Cells (ILCs) promote tissue homeostasis, contribute to the immune defense mechanisms, and play important roles in the initiation of immune responses and chronic inflammation.

Objective: To understand the roles of innate lymphoid cells in the pathophysiology of colorectal cancer (CRC) in the mouse model.

Methods: CRC was induced using azoxymethane (AOM) and dextran sulfate sodium (DSS) in Balb/c mice (the chemically induced group=18 mice), or orthotopic injection of CT-26 cell line into the colon of another set of Balb/c mice (the orthotopic group=14mice). Normal saline was injected into 18 mice, as the sham group. After 80 days, the chemically induced group was divided into two subgroups, dysplasia (8 mice) and reparative change (10 mice), based on pathological examinations. The frequencies of ILC1, 2, and 3 were then measured in colon tissues using flow cytometry by four markers including an anti-mouse lineage cocktail (FITC anti-mCD3/FITC anti-mGr-1/FITC anti-mCD11b/ FITC anti-mCD45R (B220)/FITC anti-mTer-119), PE/Cy7 anti-mouse CD45, PE anti-mouse CD117 (c-kit), and APC anti-mouse IL-33 Rα (ST2).

Results: The total ILC population was significantly higher in the chemically induced reparative change compared with the sham group. ILC1 percentage in the chemically induced reparative change was significantly higher compared to those in the other three groups (Sham, chemically induced dysplasia and orthotopic dysplasia). The orthotopic dysplasia group showed more ILC3 percentage than the other groups.

Conclusion: ILC1 and ILC3 subgroups increased significantly in reparative and dysplastic experimental CRC respectively. Thus ILC1 may have an inhibitory effect on tumor growth whereas ILC3 promotes tumor progression.

Keywords: Colorectal Cancer, CT-26, Dysplasia, Innate Lymphoid Cell, Reparative Change

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INTRODUCTION

The third most commonly diagnosed cancer among both sexes in the United States is colorectal cancer (CRC). Incidence and mortality rates have been declining for several decades because of our better understanding of risk factors, the introduction of live screening tests (i.e. colonoscopy), and the improvement of therapeutic methods [1]. It has been commonly thought that inflammation due to innate immune responses could inhibit tumor growth; however, it is now clear that tumor-associated inflammation can exert an opposite action in some cancers [2]. CRC is associated with diverse immune cell infiltrates, including innate immune cells, like macrophages, neutrophils, mast cells, and natural killer (NK) cells, as well as adaptive immune cells, such as T and B lymphocytes [2]. Within the past few years, newly identified cytokine-producing innate lymphoid cells (ILCs), characterized by a lack of antigen-specific receptors, have been shown to play important roles in immune surveillance, initiation of immune responses, tissue remodeling [3], wound healing [4], and chronic inflammation [5]. Moreover, ILC populations are currently known to be associated with the barrier tissues, such as the gastrointestinal tract, lung mucosa, and skin [6].

ILCs respond rapidly to tissue damage by secreting large amounts of cytokines, suggesting a role for these cells in the early phases of oncogenesis and in shaping the tumor microenvironment [3]. The outcome of ILC activation depends on the particular tissue environment. Each ILC subset may play a different role in the tumor progression according to the soluble factors present in the tumor microenvironment. It is due to ILC plasticity and the pleiotropic functions of the cytokines they produce [3]. Three ILC subsets are termed ILC1s, ILC2s, and ILC3s based on their ability to produce T helper 1 (Th1), Th2, and Th17 cell-associated cytokines, respectively [3]. ILCs neither express T cell receptor (TCR) nor express B cell receptor (BCR), and are defined as lineage-negative cells, whereas they possess the classic morphology of lymphoid cells [7]. ILCs also express subunits of cytokine receptors including interleukin (IL)-2 receptor-a (CD25) and IL-7 receptor-a (CD127) [7]. Nonetheless, much of the role of ILCs remains to be elucidated [8]. In addition to cytokine production, ILCs share some activation and inhibitory receptors with NK and T cells, which could be targeted in immunotherapy. A better understanding of ILC response to tumors could lead to the development of new therapeutic methods targeting these cells in the future [9]. To the best of our knowledge, no study has yet determined the alteration of ILC subsets in mouse models of CRC. Therefore, we designed this experimental study to induce CRC in Balb/c mice and evaluate the frequencies of the three main ILC subsets.

MATERIALS AND METHODS

Mice

Male Balb/c mice were provided by the Animal Institute of Mazandaran University of Medical Sciences (Sari, Iran). All mice were under 30 grams and were 6-8 weeks old. We kept the mice under controlled conditions at 23±2 ˚C in a reverse 12/12 h light/dark cycle. We divided the mice into three groups the sham, the orthotopic, and the chemically induced. The orthotopic group included 14 mice. CT-26 as a colorectal cancer cell line was orthotopically injected into the cecum wall. The sham group consisted of 18 mice and isotonic saline was injected intraperitoneally. The chemically induced group consisted of 18 mice. The study was approved by the Ethical Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1397.4665).

Induction of Colitis-associated Cancer with AOM and DSS

Eighteen mice were subjected to chemical

induction of CRC using azoxymethane (AOM) dextran sulfate sodium (DSS) in drinking bottles. On day 0, the baseline weights were recorded and each mouse of the chemically induced group was injected intraperitoneally (IP) with 15 mg/kg of AOM. Each mouse of the sham group was IP injected with isotonic saline on the same days as the chemically induced group to equalize the impact of the injection stress. A DSS cycle consists of one week of 2% (2 mg/100ml) DSS in the drinking water followed by 1 week of regular (autoclaved) water. The first DSS cycle started on day 7. On day 21, 7.5 mg/kg of AOM were IP injected, then, on day 28, the second cycle of DSS solution was done. On day 50, another dose of AOM (4 mg/kg) was injected into the chemically induced group of mice. On day 80, the establishment of CRC as the dysplasia was confirmed by a pathologist, based on the pathological scores, like cellular forms, the shape of nuclei, and lymphocyte infiltration (Figure 1).

Induction of CRC Via Orthotopic Injection of CT-26 Cell Line

CT-26, a murine colorectal cancer cell line, was obtained from the Pasteur Institute of Iran (Tehran, Iran) and cultured in RPMI-1640 medium (Biosera, Germany) enriched with 10% fetal bovine serum (FBS, Biosera, Germany) and 1% penicillin/streptomycin. Cells were maintained in culture flasks and grown in a humidified %5 CO2 at 37 ˚C. After three weeks, the cells were counted and prepared for injection. The mice were anesthetized with 40 mg/kg of ketamine-xylazine, and then the abdomen was opened with sterile scissors under a completely sterile condition. 50,000 cells were directly injected into the cecum wall. Then, the peritonea and abdomen wall were gently sewn and the mice were carefully recovered.

Cell isolation and Preparation

Sampling was done on days 80, 105, and 125. We anesthetized the mice and then euthanized them. Each mouse was then fixed and the abdominal skin was cut. We extracted the whole colon with scissors and placed dissected large intestine (mesentery removed) into a petri dish on ice with 10 ml of ice-cold phosphate-buffered saline (PBS) containing %2 FBS before opening the tissue. The inside of the lumen of the colon was washed three times carefully with normal saline, using a 5-ml syringe and 18-G blunt end needle to eradicate the bulk of fecal.

Colon tissue was cut longitudinally to expose the lumen, and then the intestines

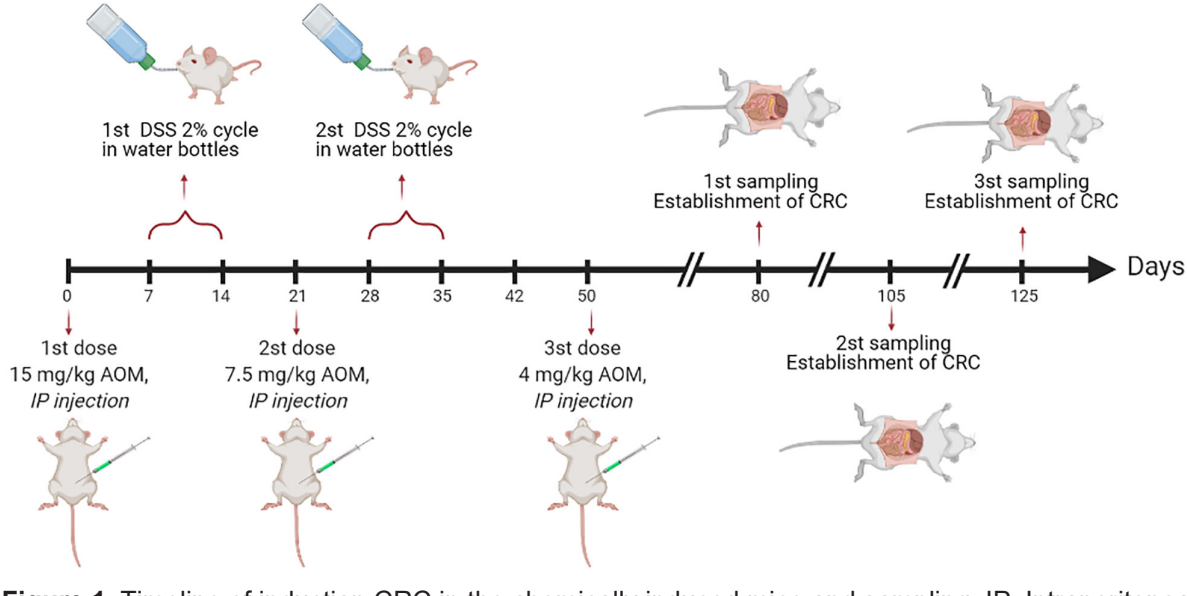

Figure 1. Timeline of induction CRC in the chemically induced mice and sampling. IP: Intraperitoneal; DSS: Dextran sulfate sodium; CRC: Colorectal cancer; AOM: Azoxymethane

were cut into 2 cm sections. We dissected luminal mucus mechanically, and placed the colon tissues into a 15-ml falcon tube with 5 ml of PBS containing %2 FBS and were vortexed to wash out the remaining fecal matter. Using a 1- to 2-mm wire mesh strainer, dirty supernatant was discarded from the intestines, and the wash was repeated three to four times. We incubated the tissues with HBSS without Ca and Mg, with FBS 2%, 2mercaptoethanole (2ME) 2mM, Ethylenediaminetetraacetic acid (EDTA) 2.7Mm at 37˚C for 60 minutes. We digested the colon tissues with collagenase IV 0.8 mg/ml at 37 ˚C for 60 minutes. Samples were homogenized using a 5-ml syringe and then were passed through a 70 µm cell strainer. Finally, the samples were washed with PBS containing %0.02 BSA buffer twice.

Pathological Examination

Three pieces of the colon, including the cecum, proximal colon, and distal colon, were fixed in 10% neutral buffered formalin. Based on the standard protocol, tissue passage was embedded in paraffin, and then the sectioning and H&E staining were done. The severity of the dysplasia was diagnosed by a pathology specialist based on scores of the morphology of colon cells and other scores based on pathology guidelines.

Flow Cytometry Analyses

Isolated cells from colon tissues were immediately stained and incubated with fluorochrome-conjugated antibodies against four surface protein markers, including an 8 µl anti-mouse lineage cocktail (FITC anti-mCD3/FITC anti-mGr-1/FITC anti-mCD11b/ FITC anti-mCD45R (B220)/FITC anti-mTer-119), 1 µl PE/Cy7 anti-mouse CD45, 1µl PE anti-mouse CD117 (c-kit), 1 µl APC-and mouse IL-33 Rα (ST2) which were incubated at 4˚C and darkness for 1 hr. All antibodies were purchased from Biolegend (California, USA). The data were recorded on a BD FACS CALIBUR (BD Biosciences, New Jersey, USA) using FACS Diva Software Version 7 and 8 (BD). The data analyses were performed using the FlowJo Software Version 8 (TreeStar).

Statistical Analyses

All the statistical analyses were performed using the Graphpad PRISM (Virginia, USA). The results were evaluated by the T-test and Kolmogorov-Smirnov tests as appropriate. The P-values of less than 0.05 were considered significant. All the graphs were designed using the Graphpad Prism 8 software. The data were expressed as means±standard error of the mean (SEM).

RESULTS

Pathological Findings

Specimens from the cecum, proximal and distal parts of the colon were examined by a pathologist. Samples from the sham group showed no pathological lesions (Figure 2A). Those from the chemically induced reparative change group represented precancerous inflammation (Figure 2B), and those from the chemically induced dysplasia represented the characteristics of the primary stages of CRC (Figure 2C).

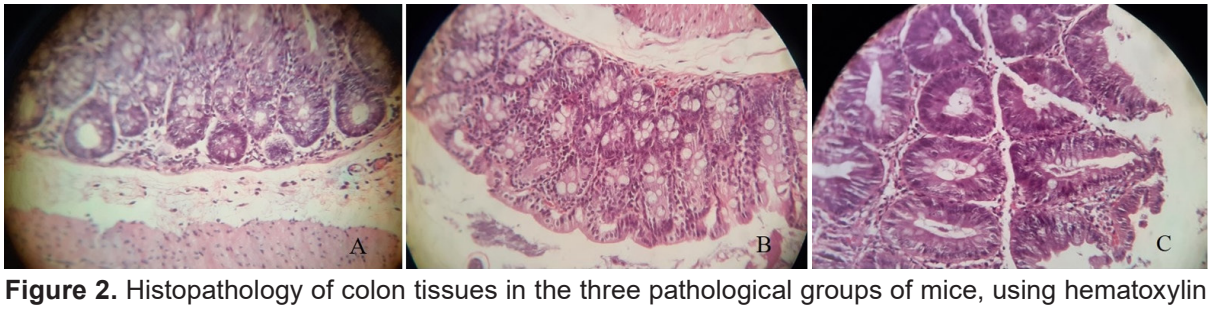

Figure 2. Histopathology of colon tissues in the three pathological groups of mice, using hematoxylin and eosin staining. A) Normal colon, B) Reparative change, C) Dysplasia. Original magnification, A, B, and C X400

Table 1. Pathological characteristics of the samples

Group name	Treatment	Total number	Pathological findings		
			Normal	Reparative change	Dysplasia
Sham	Normal saline	18	18	0	0
The Chemically-induced group	AOM + DSS	18	0	10	8
The orthotopic group	Cell line (CT-26)	14	0	0	14

DSS: Dextran sulfate sodium; AOM: Azoxymethane

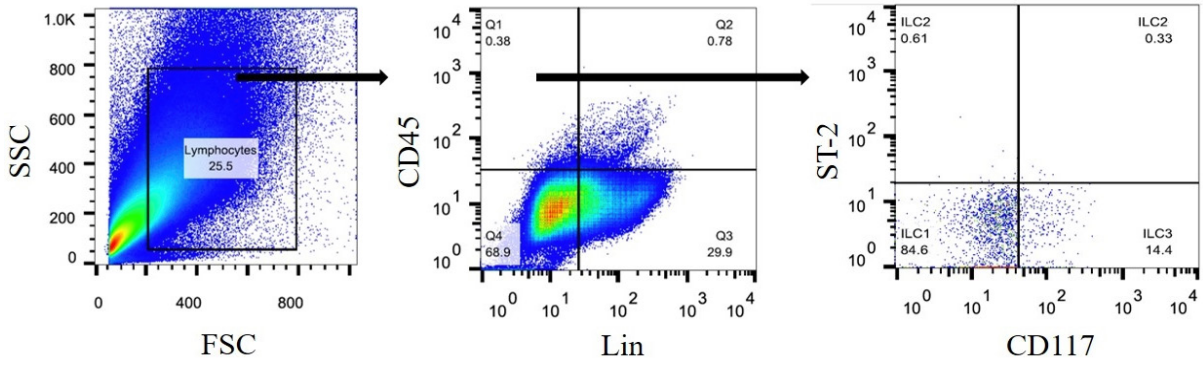

Figure 3. Frequencies of ILCs were determined using the flow cytometry. First, the population of leukocytes was gated. Total ILC population was then defined as Lin- CD45+; and ILC subpopulations were defined as ILC1 (Lin- CD45+ CD117- ST-2-), ILC2 (Lin- CD45+ CD117+/- ST-2+), and ILC3 (Lin- CD45+ CD117+ ST-2-). ILC: Innate lymphoid cell

Also, in a two-series sampling of the orthotopic group, they exhibited features of severe dysplasia (Table 1).

Total ILC Population

Frequencies of ILCs were determined using the flow cytometry (Figure 3). The total ILC population in the chemically induced reparative change mice was significantly higher than in those of the sham group (P=0.0014). The chemically induced dysplasia group showed a higher total ILC population than the sham group, although it was not statistically significant. In the orthotopic dysplasia group of mice, the results illustrated a non-significant decrease in the percentage of total ILCs compared with the sham group (Figure 4).

The ratio of ILC1/total ILC numbers in the chemically induced reparative change group was %14.7 higher than in those in the sham group (P<0.0001). As for the chemically induced dysplasia group, the difference was not significant. The orthotopic dysplasia

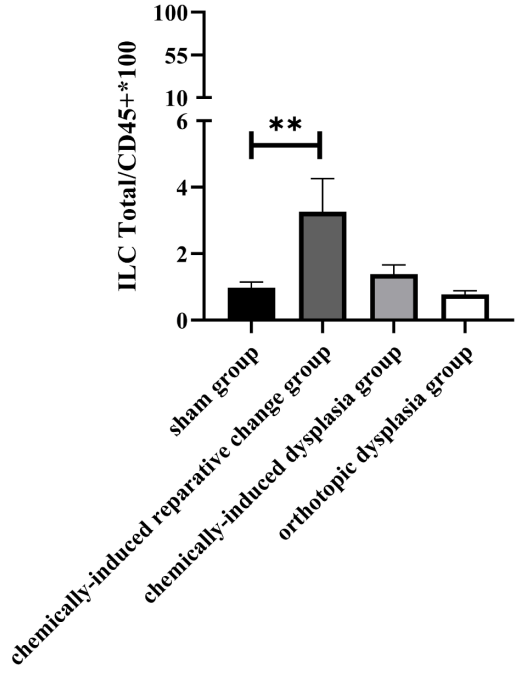

Figure 4. The mean percentage of total ILC/CD45+ events is shown in 4 pathological groups in all series of sampling. In the sham group, it was %0.97, in the chemically induced reparative change was %3.26, in the chemically induced dysplasia it was %1.3, and in the orthotopic dysplasia group it was %0.77 (**P<0.01). ILC: Innate lymphoid cell

group of mice showed a significant change compared with the sham group (P<0.0001) (Figure 5A). The ratio of ILC2/total ILC numbers was not significantly different in all three pathological groups compared with those in the sham group (Figure 5B). The frequencies of the ILC3 population in the chemically induced reparative change mice showed significant alteration compared with those in the sham group (P<0.0001). The results for the chemically induced dysplasia illustrated no significant change compared with those in the sham group. In contrast, the difference between the orthotopic dysplasia group and the sham group was significant (P<0.0001, Figure 5C).

Distribution of ILCs

In all pathological groups, ILC1s were the highest (more than 50%) ILCs, while ILC2s were the least (less than 5%). The distribution of these cells in the orthotopic and the chemically induced reparative change group altered a lot in comparison with those in the sham group (Figures 6B and 6D), while the frequencies of ILCs in the sham and the

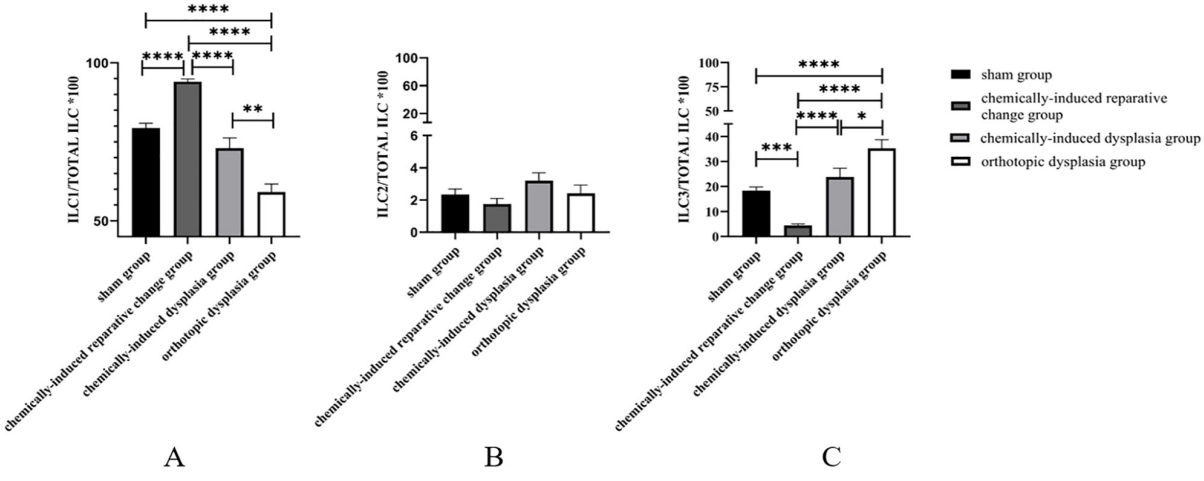

Figure 5. The alterations in ILC subpopulations in 4 pathological groups. A) The mean ratio of ILC1/total ILCs was %79.3 in the sham group, %94 in the chemically induced reparative change, %75 in the chemically induced dysplasia, and %60 in the orthotopic dysplasia group. B) The mean ratio of ILC2/total ILCs was %2.34 in the sham group, %1.75 in the chemically induced reparative change group, %3.195 in the chemically induced dysplasia group, and %2.41 in the orthotopic dysplasia group. C) The mean ratio of ILC3/total ILCs was %18.3 in the sham group, %4.4 in the chemically induced reparative change group, %23.7 in the chemically induced dysplasia group, and %35.5 in the orthotopic dysplasia group was just under *P<0.05, **P<0.01, ***:P<0.001, and ****P<0.0001. ILC: Innate lymphoid cell

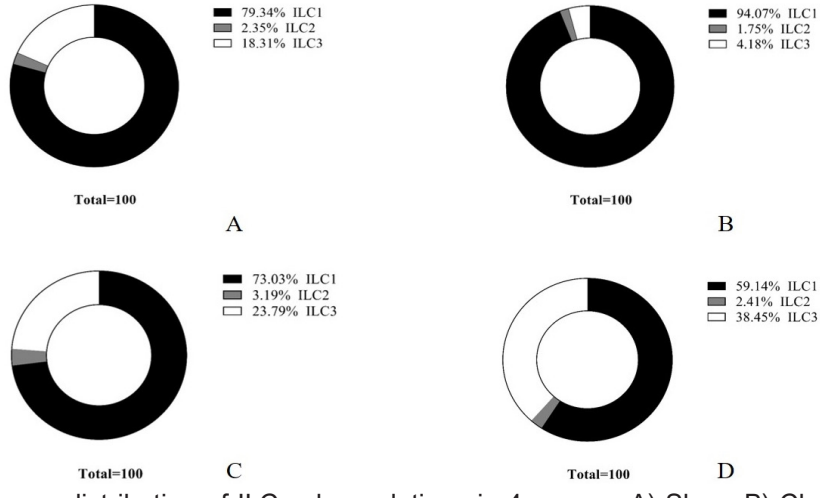

Figure 6. The mean distribution of ILC subpopulations in 4 groups. A) Sham B) Chemically induced reparative change group, C) Chemically induced dysplasia group, and D) Orthotopic dysplasia group. ILC: Innate lymphoid cell

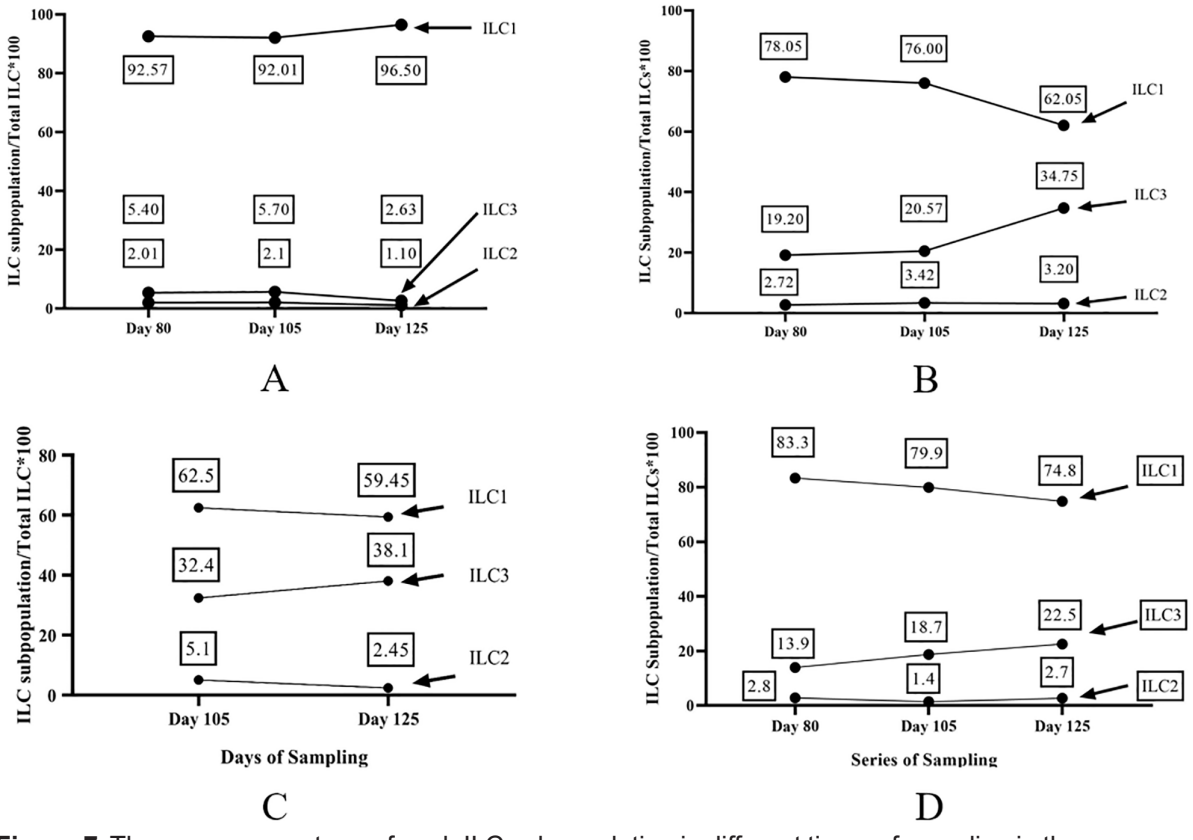

Figure 7. The mean percentage of each ILC subpopulation in different times of sampling in three groups, A) Chemically induced reparative change group, B) Chemically induced dysplasia group, C) Orthotopic dysplasia group, D) Sham group. ILC: Innate lymphoid cell

chemically induced dysplasia group are close to each other (Figures 6A and 6C).

Alterations in Three Series of Sampling

Although the percentage of populations of ILCs in three series of sampling in the sham group and the chemically induced reparative change group was almost stable (Figures 7A and D: however, these amounts were not stable during the days of sampling in the chemically induced dysplasia group (Figure 7B). The presence percentage of ILC1s during three days of sampling (80, 105, and 125) was declining, so that on day 80 it was slightly lower than 80 percent, and on day 125 it reached a little more than 60 percent; while this percentage and concerning ILC3s was increasing, somehow on the first day of sampling it represented almost 20 percent, and the more days spent, the more percentage of this subpopulation were seen. On the last day of sampling, it reached close to 40% (Figure 7B). On the other hand, during the two series sampling of the orthotopic dysplasia group of mice the frequency of ILC1 fell from %62.5 to %59.45, while ILC2 altered slightly from 5.1 percent to %2.45, and ILC3 experienced an increase from %32.4 to %38.1 (Figure 7C).

DISCUSSION

The findings of the current study illustrate alterations in ILC subpopulations in CRC. Generally, these changes include increased ILC1 percentage in the reparative change group from the chemically induced mice and increased ILC3 percentage in the dysplasia group from both the chemically induced and the orthotopic dysplasia groups.

It had been shown that in the inflamed colon tissues in patients with Crohn's disease, there was an increased frequency of ILC1 lacking NK cell-specific markers, which secret IFN-γ. Also, the higher frequency of ILC1 in the inflamed colon of mice was

reported as a direct impact of inflammation. These findings suggest an essential role by IFN-γ producing ILC1s in colon inflammation [10]. On the other hand, via RNA sequencing of tumor-infiltrated ILCs in a mouse model of CRC, it was demonstrated that at the end stages of CRC, ILC1s could act as immune inhibitors via expressing inhibitory receptors [11]. Another study showed a decrease in total ILC numbers in tumor tissues of CRC patients, while the ILC1 number elevated [12]. During the tumor progression, ILC1s undergo changes leading to immune inhibitory functions in the late stages of cancer. As an example, in the primary stages of CRC, ILC1s and NK cells produce large amounts of IFN-γ, but as the tumor progresses, those cells are influenced by the inflammatory microenvironment and produce relatively less amount of that cytokine. This could in turn lead to a decreased immune surveillance and finally tumor progression [11].

In the present study, it was observed that in the reparative change group from the chemically induced mice, ILC1s accounted for a significant percentage of ILCs. Therefore, we assumed that these cells probably play an important role in the induction of the inflammatory microenvironment by producing cytokines, like IFN-γ and TNF-α. These cytokines act similarly as a double-edged sword so that they could either inhibit the tumor progression by increasing the immune cell trafficking into the tumor environment, inducing apoptosis directly in tumor cells, or provide a condition for the growth and proliferation of cancer cells via the induction of chronic inflammation [9]. ILC1s express both inhibitory (PD-1 and CTLA-4) and activation (TRAIL and NKG2D) markers, so they can inhibit or activate themselves and other immune cells for and against tumor cells, respectively [9]. Taken together, ILC1 seems to have both immunostimulatory and immunoinhibitory functions in different cancers.

We did not observe significant changes among the four groups regarding the ILC2s. A previous study showed a significant increase in the numbers of ILC2s in PBMCs from gastric cancer patients. Furthermore, the expression of ILC2-related genes, including RORα, GATA3, T1/ST2, IL-17RB, CRTH2, IL33, IL4, and IL5 were higher in those patients compared with those in the healthy subjects [13]. Another study indicated that the expression of IL-33 and ST-2, related to ILC2 function, was low in the early stages of CRC in both human subjects and AOM/DSS-induced mouse model, while they were highly expressed in the late stages of cancer. Moreover, the mice lacking the ST2 gene showed resistance against tumor development. Also, further activation of this pathway resulted in the epithelial barrier weakening in the colon and the induction of immune cells to release the tumorigenic cytokine, IL-6. Finally, this pathway can act as a trigger for tumorigenesis [14]. In parallel to the current study, a study done on CRC patients suggested that the ILC2 number was the lowest among the ILC subsets and that it was stable during the tumor progression [15].

Some studies state an immunomodulatory role for ILC2 [16]. What is more, some studies have shown that the number of these cells was higher in the gut malignancies [11, 17]. Increased ILC2 number plus IL-13 produced by these cells can induce and activate MDSCs; it can also lead to an immune-suppressive state of the tumor microenvironment [13]. In the present study, the frequency of this subpopulation in dysplasia groups both in the chemically induced and orthotopic mice was higher than those in the control mice. This indicates the effective presence of these cells in pathogenic circumstances and more developed states of cancer. This is probably explainable by the IL-33/ST-2 and IL-13/MDSC pathways discussed in former studies [18, 19]. Nevertheless this needs to be further investigated.

ILC2s are the smallest ILC subpopulation in colon tissue. These cells are always referred to as Th2 counterparts. The induction of type II immune responses, including tissue repair,

activity against parasite pathogens, tissue fibrosis, eosinophil activation, and allergic responses, are the major functions of ILC2s similar to Th2 cells [9]. These cells produce IFN-γ and IL-13 and play important roles in CRC development. IFN-γ produced by ILC2s acts similar to what is explained about ILC1. In our study, although ILC2 frequency was slightly higher in both the dysplasia groups compared with two other groups of mice, they were not statistically significant. These findings might demonstrate the activity of these cells in favor of the tumor progression.

Regarding the ILC3 population, we showed higher frequencies of ILC3s in tumor tissue of the colon than in those in normal tissue. A previous study, in a mouse model of CRC, revealed elevated levels of IL-17 and IL-23 from ILC3 which were associated with the tumor progression. This has also been shown in some human cancers, like breast, ovarian, liver, pharyngeal, skin, and gastric cancers. Therefore, ILCs might be related to tumorigenesis, by providing an inflammatory microenvironment in favor of the tumor [20].

ILC3s are activated via IL-23 and can inhibit the cytotoxicity of T and NK cells, which could provide an appropriate condition for tumor growth. On the other hand, via producing IL-2 and IL-8, ILC3s could promote leukocyte infiltration into the tumor niche to stop the growth and development of the tumor. They also express NKP-44 and NKP-46, similar to NK cells, which make them active [9]. Diversely, they fulfill an essential role in CRC progression via expressing the immunomodulatory cytokine, TGF-β [11]. Our study showed higher frequencies of ILC3 in both chemically induced and the orthotopic dysplasia groups, which are representative of cancerous states, compared with the other group. This indicates the possible prominent role of the pro-tumorigenic function of these cells.

Our results also showed higher ILC1 and lower ILC3 numbers in the chemically induced reparative change group, representing precancerous inflammation, compared with those of the sham group. Concurrently, a previous study found decreased ILC3s in inflamed tissues in patients suffering from Crohn's disease and ulcerative colitis compared with those in non-inflamed tissues from the same patients [21]. Another study reported fewer total ILC numbers and ILC3 numbers in tumor tissues from patients with CRC [12] which is in contrast with the results of the present study. The probable reasons for this contradiction are sample discrepancy, the evaluation of the pre-cancer and cancer status simultaneously in comparison with the normal samples.

CONCLUSION

ILC1 and ILC3 subgroups increased significantly in reparative and dysplastic experimental CRC respectively. Thus ILC1 may have an inhibitory effect on tumor growth and ILC3 prompts tumor progression .So we recommended ILC conversion in experimental CRC immunotherapy.

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Conflict of Interest: None declared.

REFERENCES

1. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. CA: a cancer journal for clinicians. 2017;67(3):177-93.
2. Grizzi F, Bianchi P, Malesci A, Laghi L. Prognostic value of innate and adaptive immunity in colorectal cancer. World journal of gastroenterology. 2013;19(2):174-84.

3. Moro K, Ealey KN, Kabata H, Koyasu S. Isolation and analysis of group 2 innate lymphoid cells in mice. Nature protocols. 2015;10(5):792-806.
4. Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, et al. Innate Lymphoid Cells: 10 Years On. Cell. 2018;174(5):1054-66.
5. Simoni Y, Fehlings M, Kloverpris HN, McGovern N, Koo SL, Loh CY, et al. Human Innate Lymphoid Cell Subsets Possess Tissue-Type Based Heterogeneity in Phenotype and Frequency. Immunity. 2018;48(5):1060.
6. Halim TY, Takei F. Isolation and characterization of mouse innate lymphoid cells. Current protocols in immunology. 2014;106:3.25.1-13.
7. Artis D, Spits H. The biology of innate lymphoid cells. Nature. 2015;517(7534):293-301.
8. Vivier E, Vely F, Fischer A. Reply to 'Comment on: Evidence of innate lymphoid cell redundancy in humans'. Nature immunology. 2018;19(8):789-90.
9. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. Nature reviews Immunology. 2018;18(11):671-88.
10. Bernink JH, Peters CP, Munneke M, te Velde AA, Meijer SL, Weijer K, et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. Nature immunology. 2013;14(3):221-9.
11. Wang S, Qu Y, Xia P, Chen Y, Zhu X, Zhang J, et al. Transdifferentiation of tumor infiltrating innate lymphoid cells during progression of colorectal cancer. Cell research. 2020.
12. Carrega P, Orecchia P, Quatrini L, Tumino N, Venè R, Benelli R, et al. Characterisation of innate lymphoid cell subsets infiltrating colorectal carcinoma. Gut. 2020.
13. Bie Q, Zhang P, Su Z, Zheng D, Ying X, Wu Y, et al. Polarization of ILC2s in peripheral blood might contribute to immunosuppressive microenvironment in patients with gastric cancer. Journal of immunology research. 2014;2014:923135.
14. Mertz KD, Mager LF, Wasmer MH, Thiesler T, Koelzer VH, Ruzzante G, et al. The IL-33/ST2 pathway contributes to intestinal tumorigenesis in humans and mice. Oncoimmunology. 2016;5(1):e1062966.
15. Carrega P, Orecchia P, Quatrini L, Tumino N, Venè R, Benelli R, et al. Characterisation of innate lymphoid cell subsets infiltrating colorectal carcinoma. Gut. 2020.
16. Atreya I, Kindermann M, Wirtz S. Innate lymphoid cells in intestinal cancer development. Seminars in immunology. 2019:101267.
17. Mattner J, Wirtz S. Friend or Foe? The Ambiguous Role of Innate Lymphoid Cells in Cancer Development. Trends in immunology. 2017;38(1):29-38.
18. Larsen KM, Minaya MK, Vaish V, Peña MMO. The Role of IL-33/ST2 Pathway in Tumorigenesis. International journal of molecular sciences. 2018;19(9).
19. Zhao N, Zhu W, Wang J, Liu W, Kang L, Yu R, et al. Group 2 innate lymphoid cells promote TNBC lung metastasis via the IL-13-MDSC axis in a murine tumor model. International immunopharmacology. 2021;99:107924.
20. Elif Saritas Yuksel1, Firdevs Topal1, Özlem Gür2, Fatih Topal1, Cengiz Karahanlı1, Uran2 B. IL-17 and IL-22 in colitis associated colorectal cancer in mice. Medical Science. 2019.
21. Forkel M, van Tol S, Hoog C, Michaelsson J, Almer S, Mjosberg J. Distinct Alterations in the Composition of Mucosal Innate Lymphoid Cells in Newly Diagnosed and Established Crohn's Disease and Ulcerative Colitis. Journal of Crohn's & colitis. 2019;13(1):67-78.