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How CD300e affects tumor-associated macrophages in colon cancer

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To my family – for believing in me, even when I didn't believe in myself, and for their constant support throughout this journey.

To my best friend, without whose encouragement I might never have enrolled in this degree.

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ABSTRACT

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide and remains difficult to treat due to its genetic heterogeneity and a complex tumor microenvironment. Recent studies have focused on the role of tumor-associated macrophages (TAMs) in cancer progression and how their behaviour can be altered to improve treatment outcomes. One molecule of interest is CD300e, a receptor found on myeloid cells. Although CD300e typically activates immune responses, recent studies suggest that its activation in monocytes reduces the expression of antigen presentation molecules, which are essential for antigen presentation to T cells, meaning that CD300e activation may limit T cell mediated responses rather than promote them under certain conditions.

To investigate the role of CD300e in CRC, we used a subcutaneous mouse model in wild-type (WT) and CD300e knockout (KO) mice. Tumors were induced by injecting MC38 cells and tumor growth was monitored over 21 days. At the end of the study, tissue and blood samples were collected for further analysis. We found that macrophages from CD300e KO mice had a higher phagocytic activity compared to those in WT mice, suggesting that CD300e may influence macrophage activity within the tumor environment. However, both groups showed similar tumor weight and volume at the time of the sacrifice, which was confirmed by statistical tests. These findings support the previous conclusion that CD300e promotes immune suppression by altering macrophage behaviour in tumors. Therefore, blocking CD300e could help enhance anti-tumor immune responses and improve CRC treatment.

1. INTRODUCTION

1.1. Colorectal cancer

Colorectal cancer (CRC), which includes malignancies of the colon and rectum, is the second most common cause of cancer-related deaths worldwide, accounting for 9.4% of cancer-related mortality in 2020[1]. Due to its high rates of incidence and mortality, CRC is a significant global public health concern[2]. In fact, the number of CRC cases is expected to reach 3.2 million and cause 1.6 million deaths per year by 2040[1]. While CRC used to be prevalent in older adults, its incidence is now rising among individuals under the age of 50[2].

The development of CRC is associated with both nonmodifiable risk factors, such as age and genetic predisposition, and modifiable environmental and lifestyle factors. Chronic inflammatory conditions like inflammatory bowel disease (IBD) significantly increase CRC risk. Compared to the general population, individuals with IBD are approximately twice as likely to develop CRC. This is primarily due to persistent inflammation in the intestinal tract, which results in the abnormal release of cytokines, further promoting carcinogenesis. The association between ulcerative colitis and CRC is well-documented, while the link between Crohn's disease and CRC remains less clear[2]. Obesity is another important risk factor for CRC. Adipocytes within the tumor microenvironment not only provide energy that supports cancer cell growth but also contribute to the secretion of adipokines and proinflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α). These molecules can activate signalling pathways that promote tumor proliferation and metastasis. Diet is another modifiable factor influencing CRC risk. Pro-inflammatory diets high in carbohydrates, proteins, and trans fats have been associated with an increased risk of CRC. In contrast, foods rich in fiber, unsaturated fatty acids, and fat-soluble vitamins have anti-inflammatory properties and are linked to a lower risk of CRC. Other established risk factors include alcohol consumption, smoking, and diabetes, with diabetes increasing CRC risk independently of body weight or physical activity. Early detection through screening of high-risk individuals, along with modifying certain risk factors, such as physical inactivity, obesity, smoking, and alcohol consumption, could improve patient outcomes[2].

CRC development involves a series of biological processes, such as abnormal cell proliferation, resistance to apoptosis, and the spread of cancer cells to nearby tissues and organs. Various genes and

signalling pathways are involved in the onset of CRC, but the exact mechanisms are not yet fully understood. Most cases of CRC are sporadic and develop slowly over many years. The development and progression of this disease is due to the accumulation of mutations in key signalling pathways, such as Wnt, epidermal growth factor receptor (EGFR), P53, and transforming growth factor beta (TGF-β)[2].

The complexity of CRC, including its genetic heterogeneity and tumor microenvironment, makes it difficult to develop effective treatments. Given this, preclinical models are crucial in cancer research as they allow the scientists to study tumor biology, test new therapies, and predict clinical outcomes before human trials. In vitro cancer cell lines provide valuable insights into cancer mechanisms and drug responses but lack the full complexity of human tumors. Furthermore, the repeated in vitro culturing leads to genetic and epigenetic changes over time. To overcome these limitations, in vivo tumor models using the MC38 cell line are often used. The MC38 cell line is a murine colon adenocarcinoma model widely used for studying CRC[3].

1.2. Macrophages in Colorectal Cancer

Tumor-associated macrophages (TAMs) are one of the most common immune cells found in the tumor microenvironment (TME), making up approximately 30-50% of all immune cells present[5,4]. The TME is a complex environment made up of tumor cells, blood vessels, immune cells, fibroblasts, signalling molecules, and the extracellular matrix. It plays a major role in cancer progression by supporting tumor growth and weakening the immune response[5].

TAMs originate from two main sources: tissue-resident macrophages (TRMs) and circulating monocytes[5]. TRMs are formed early in development from embryonic precursors in the yolk sac or fetal liver and survive in the adulthood without the help of new blood monocytes[6]. During tumor development, monocytes from the blood are recruited to the tumor site by chemotactic signals produced by tumor cells and surrounding tissues. Once they arrive in the TME, these monocytes are exposed to signals like macrophage colony-stimulating factor (M-CSF), TGF-β, and various chemokines. These signals influence the monocytes to differentiate into macrophages, which can assume many different functional profiles, from the immunostimulatory to the immunosuppressive, depending on the cytokines present in the TME[6].

Immunostimulatory macrophages, also known as classically activated macrophages, are pro-inflammatory and have antitumor activity. They are activated by signals such as interferon-γ (IFN-γ) and TNF-α, and they release cytokines like IL-1β, IL-6, and IL-12, which help enhance immune responses and inhibit tumor growth[5,6]. Immunostimulatory macrophages express surface markers such as CD86 and CD64 and are highly phagocytic and cytotoxic[7]. Immunosuppressive macrophages, also known as alternatively activated macrophages, are induced by cytokines such as IL-4, IL-13, and TGF-β and normally promote tissue repair, angiogenesis, and immune suppression. However, they are often found within tumors, where these same functions are used to support tumor progression[6]. Immunosuppressive macrophages express surface markers such as CD36, CD206, and CD163 and are less effective at presenting antigens to T cells[6].

TAMs also demonstrate plasticity, meaning they can switch between immunostimulatory and immunosuppressive types depending on local environmental signals. Immunostimulatory macrophages are typically found in early stages of tumor development, but as the tumor develops, cytokines present in the TME gradually shift them toward the immunosuppressive phenotype[5]. A high immunosuppressive/immunostimulatory ratio is usually associated with poor prognosis[6].

In the classical antigen presentation process, immunostimulatory macrophages support the immune response by increasing the expression of molecules such as MHC-II, CD80, and CD86 on their surface. These molecules are important because they help activate T cells. They phagocytose tumor antigens, break them down in lysosomes, and then present the antigen peptides on their surface as MHC-II-antigen complex. T cells recognize these complexes through their receptors (TCRs), which activates them to fight the tumor. However, when T cells become exhausted, they release signals that attract more monocytes. These monocytes develop into TAMs, which keep presenting antigens and activating T cells. This makes the T cells even more exhausted and weakens the immune response[5].

TAMs promote cancer in many ways. They help tumor cells grow, spread to other parts of the body, and form new blood vessels. TAMs release numerous reactive oxygen and nitrogen species, which can damage DNA and increase mutation rates, leading to genomic instability and higher cancer risk. In addition, TAMs secrete factors like TNF-α, TGF-β, and IL-1β, which enable cancer cells to break through tissue barriers, helping cancer cells invade nearby tissues. TAMs also play a role in a process called epithelial-mesenchymal transition (EMT). In studies on colon cancer, TAMs were found to

trigger EMT through a specific signalling pathway. This made the tumor cells more invasive and attracted even more TAMs to the tumor, creating a cycle that makes the cancer worse[5]. TAMs also promote the formation of new blood vessels (angiogenesis), which tumors need to grow. Higher numbers of TAMs are strongly linked to more blood vessel growth in tumors. Moreover, TAMs influence tumor metabolism, especially under low-oxygen (hypoxic) conditions. Hypoxia in the TME reduces glucose uptake by TAMs, leaving more glucose available for tumor cells. This promotes aerobic glycolysis, helping tumors grow and spread[5].

An important role of TAMs is helping tumors escape immune detection. Normally, macrophages help initiate immune responses, but TAMs in tumors suppress immune activity instead. They interfere with cytotoxic T lymphocytes (CTLs), especially CD8+ T cells, which are crucial for killing cancer cells. TAMs contribute to tumor development by releasing anti-inflammatory cytokines like IL-10 and TGF-β, inhibiting T cell activation, and promoting regulatory T cells (Tregs)[6].

Targeting TAMs has become an important focus in CRC therapy. To start with, TAMs can be reprogrammed from a tumor-promoting type to a tumor-fighting type. This is possible because TAMs are flexible and can change their behaviour in response to signals from their surroundings. Since immunosuppressive macrophages restrain immune responses by releasing anti-inflammatory molecules, therapies that shift them towards the immunostimulatory phenotype could improve immune responses against tumors. Furthermore, tumor cells release cytokines and chemokines that attract TAMs to the tumor site. Blocking these signals using monoclonal antibodies could reduce the number of TAMs in the tumor and reduce their harmful effect[8].

Immunotherapy is also being studied as a way to target TAMs in CRC. In patients with high microsatellite instability, a protein called PDCD1 (also known as PD-1) is found at high levels in immunosuppressive macrophages in tumor areas. PDCD1 is also highly expressed in immnostimulatory macrophages, where it reduces their ability to destroy cancer cells through phagocytosis. Blocking PDCD1 with immune checkpoint inhibitors can restore macrophage function and improve patient survival, meaning that TAMs can be directly targeted with these therapies[9].

In addition to PD-1, recent findings suggest that CD300e may also function as an immune checkpoint molecule. Although CD300e is typically seen as an activating receptor, it has been shown to impair

antigen presentation by reducing the expression of STAT1 (Signal Transducer and Activator of Transcription 1). This limits the ability of macrophages to activate T cells, potentially weakening the overall immune response. These findings highlight CD300e as a potential new target for immunotherapy, especially in the context of enhancing macrophage-mediated anti-tumor responses[10].

1.3. CD300e

CD300e is a member of the CD300 receptor family, and its gene is located on chromosome 17q25 in humans. It is expressed at low levels in tissues such as the lung, kidney, muscle, and adipose tissue, while its highest expression is found in the bone marrow[11]. CD300e is a receptor found on the surface of myeloid cells, including monocytes, macrophages and myeloid dendritic cells. It plays an important role in regulating immune responses, such as inflammation, oxidative stress, and tissue damage and repair. CD300e activation triggers the production of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α, which contribute to immune reactions and tissue damage. Furthermore, it leads to the production of reactive oxygen species (ROS), further promoting oxidative stress and inflammation[11].

The CD300 receptor family includes both activating and inhibitory members. These receptors are type I transmembrane proteins with a single extracellular immunoglobulin-like (Ig-like) domain. In humans, there are eight CD300 family receptors (CD300a-h), expressed primarily on myeloid and lymphoid cells[10]. Some members, like CD300a and CD300f, have inhibitory functions and contain immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domains. In contrast, receptors like CD300b, CD300c, CD300d, and CD300e have transmembrane domains with charged amino acid residues, such as lysine, which allow them to associate with adaptor proteins, contributing to immune activation[12].

CD300e is typically considered an immune-activating receptor, however, its effects can be more complex. For example, when CD300e is activated in monocytes, it impairs the expression of HLA class II molecules, which are crucial for antigen presentation to T cells. This occurs because CD300e activation interferes with the function of STAT1, a protein necessary for HLA-II synthesis. As a result, CD300e-activated monocytes have a reduced ability to activate T cells through antigen presentation. This observation suggests that CD300e may have two roles: promoting immune activation and regulating T cell responses, depending on the context and the type of immune cell involved[10].

2. METHODOLOGY

2.1. MC38 Cell Culture

The experiments used the MC38 cell line, a murine colon adenocarcinoma model derived from C57BL/6J mice that is commonly used for colorectal cancer research. Cells were stored in liquid nitrogen and thawed in a 37°C water bath. Pre-warmed expansion medium consisting of DMEM high glucose, 10% FBS, and Penicillin/Streptomycin (PenStrep) was gradually added to the cells, which were then centrifuged at 300 g to remove residual DMSO, which is toxic to cells. The resulting pellet was resuspended in fresh expansion medium and transferred into a T25 flask with additional medium to ensure sufficient nutrients for initial growth. Cells were incubated at 37°C with 5% CO_2 in a humidified incubator to allow for expansion.

After several days of growth, cells were passaged using 0.25% trypsin in PBS. Cells were then centrifuged at 300 g for 5 minutes, resuspended in fresh medium and transferred into a T75 flask containing additional medium to allow for further expansion. The cells were incubated at 37°C with 5% CO_2 to promote reattachment and growth.

To determine cell concentration, a 1:100 dilution was prepared using Trypan Blue and 10 µL of the diluted cell suspension were loaded onto a Burker counting chamber.
The required volume of cell suspension was calculated in order to seed 1 million cells in the flask. After counting, an appropriate volume of the cell suspension was added to a culture flask with fresh medium. The cells were incubated at 37°C with 5% CO_2 to promote further growth.

2.2. Animal Model:

This study used C57BL/6J mice to examine the role of the CD300e receptor. Two strains were used: wild-type ($CD300e^{+/+}$) and knockout (KO) ($CD300e^{-/-}$). Mice were kept on a 12-hour light/dark cycle with free access to food and water.

2.3. Tumor Induction and Monitoring

MC38 cells were injected into six female mice, of which three wild-type (WT) and three CD300e knock-out (KO). Each mouse received a subcutaneous injection in the flank with 1×10^6 MC38 cells resuspended in 100 µl of solution composed by 50 µl PBS and 50 µl Matrigel.

The experiment lasted 21 days, during which tumor growth was monitored. Starting on day 7, tumor size was measured every two days using a caliper to track progression. A specific formula was used to calculate the tumor area: $(L \times l^2)/ 2$, where L is the larger dimension and l is the smaller one.

2.4. Tumor Dissociation and Cell Preparation

To obtain the tumours, mice were open longitudinally, and tumours were detached and collected in PBS. In order to dissociate the tumours, they were placed in petri dishes containing 5 mL of digestion buffer composed of DMEM high glucose supplemented with 1 mg/mL DNase I and 1 mg/mL Collagenase IV and cut into small pieces. The fragmented tissue was then transferred into gentleMACS™ C tubes (Miltenyi).

The C tubes were placed upside down in the Octo Dissociator machine (Miltenyi), and the 37C_M_TDK_1 program was run to further breakdown the tissue. After completion of the dissociation process, the obtained cell suspension was transferred into a Falcon tube through a cell strainer to remove undigested debris medium.

After filtration, the cell suspension was centrifuged at 300 g for 10 minutes. After centrifugation, the supernatant was removed, and the pellet was resuspended in an appropriate volume of medium based on its size. Cell counting was performed using trypan blue at a 1:100 dilution.

2.5. Macrophage Isolation

Magnetic labelling was used to selectively attach antibody-coated magnetic particles (microbeads) to macrophages in the sample. First, the cell number was determined, and the cell suspension was centrifuged to obtain a pellet. After removing the supernatant, the cell pellet was resuspended in PBS containing 0.5% BSA and 2mM EDTA. Anti-F4/80 MicroBeads UltraPure, which are coated with antibodies against the F4/80 surface marker on macrophages, were then added to the suspension, and the mixture was incubated in the refrigerator for 15 minutes in the dark. After incubation, the cells were washed by adding the same buffer and centrifuged again to remove any unbound microbeads. The cells were then resuspended in buffer for magnetic separation.

For magnetic separation, a LS MACS® (Miltenyi) column was chosen based on the number of cells to be separated. The column was prepared by rinsing with buffer, and the cell suspension was applied

onto it. Unlabelled cells passed through the column, and additional washing steps were performed to collect this flow-through. Afterward, the column was removed from the magnetic field, and the magnetically labelled cells were flushed out using a plunger and collected in a separate tube. To increase the purity of F4/80 cells, a second round of magnetic separation was performed by repeating the magnetic separation procedure by using a new column.

2.6. Macrophage Phagocytosis Assay

Macrophages isolated from the tumor were plated at a density of 50,000 cells per well in duplicate wells containing sterile glass cover slips and allowed to adhere overnight in RPMI medium supplemented with 10% FBS at 37°C with 5% CO_2. The following day, the medium was removed, and the cells were washed with PBS to remove residual medium. Subsequently, 500 µL of starvation medium - DMEM supplemented with 2% FBS - was added to each well. Plates were then incubated on ice for 30 minutes to standardize starvation conditions.

To assess phagocytosis, macrophages were exposed to fluorescent Texas Red-conjugated Zymosan A (*Saccharomyces cerevisiae*) BioParticles, which are recognized and internalized by phagocytic cells. Then, in a separate falcon tube, a mixture of starvation medium and zymosan A particles was prepared and added to the macrophages. The cells were incubated for one hour at 37°C with 5% CO_2 to allow phagocytosis to occur.

Following incubation, the medium containing the particles was removed, and the cells were washed with PBS to eliminate any unbound beads which were not engulfed by macrophages. To ensure that only internalized particles were measured, extracellular fluorescence was quenched by incubating the samples with 500 µL of 0.2% trypan blue in PBS per well for 3 minutes. This was followed by three washings with PBS to remove any residual trypan blue. To preserve the cells and stop further phagocytosis, they were fixed using a 4% paraformaldehyde solution. The fixation process occurred at room temperature for 20 minutes before the paraformaldehyde was removed. The cells were then washed three times with PBS to remove any remaining fixative. Finally, PBS was added to each well to keep the cells stable, and the plate was stored at 4°C until further use.

2.7. Immunofluorescence:

To permeabilize phagocytic macrophages, PBS was first removed from all samples, and 500 µL of 0.5% Triton-X100 in PBS was added to each well and incubated 1 hour at room temperature. After incubation, the permeabilization solution was removed, and 500 µL of 3% BSA in PBS was added to each well to block nonspecific binding. Samples were incubated for 30 minutes at room temperature, followed by a wash with PBS.

For primary antibody staining, a solution containing 1% BSA, 0.1% Triton-X100 in PBS, and the rat anti-mouse F4/80 primary antibody (non-conjugated) was prepared. Cover slips with macrophages were incubated with this mixture overnight at 4°C in a humidified chamber to allow antibody binding.

The next day, the cover slips were returned to their original wells and washed twice with PBS to remove any unbound primary antibodies. A secondary antibody mixture was prepared, containing chicken anti-rat Alexa Fluor 488 (to label macrophages green), 1% BSA, 0.1% Triton in PBS, and DAPI (for nuclear staining). Cover slips were incubated with this mixture for 1 hour at room temperature in a humidified chamber.

After incubation, the cells were washed with PBS and mounted on glass slides using mounting medium. Slides were left to dry at room temperature for 24 hours before imaging.

2.8. Confocal Microscopy

Confocal microscopy (ZEISS LSM900 Airyscan2) was used to obtain high resolution images by producing a point source of light and rejecting out-of-focus light. To visualize the samples, a 40X objective was used. Images were acquired as Z-stack to capture multiple focal planes. For analysis, a single image was processed using ImageJ (Fiji) with the maximum intensity projection setting.

3. RESULTS AND DISCUSSION

3.1. Tumor Growth Data

Tumor size in WT and KO mice was monitored three times per week with a digital caliper once the tumors were palpable – one week after cell engraftment – till sacrifice at day 21 post-injection. Tumor volume was calculated using the formula (L x l^2)/2 and reported in Fig.1a. In the graph, each dot represents the average tumor volume for each group at a specific time. Tumor volume increased progressively over time in both groups. Initially, tumors in WT mice appeared slightly larger, but over time, the tumor growth rates in both groups became similar, with no statistically significant differences between them. At the end of the protocol, mice were sacrificed and the tumors were excised (see image of isolated tumors in Fig 1b). Tumors were weighted and their final volume was calculated as H x W x L, with H, W and L being height, width and length respectively.

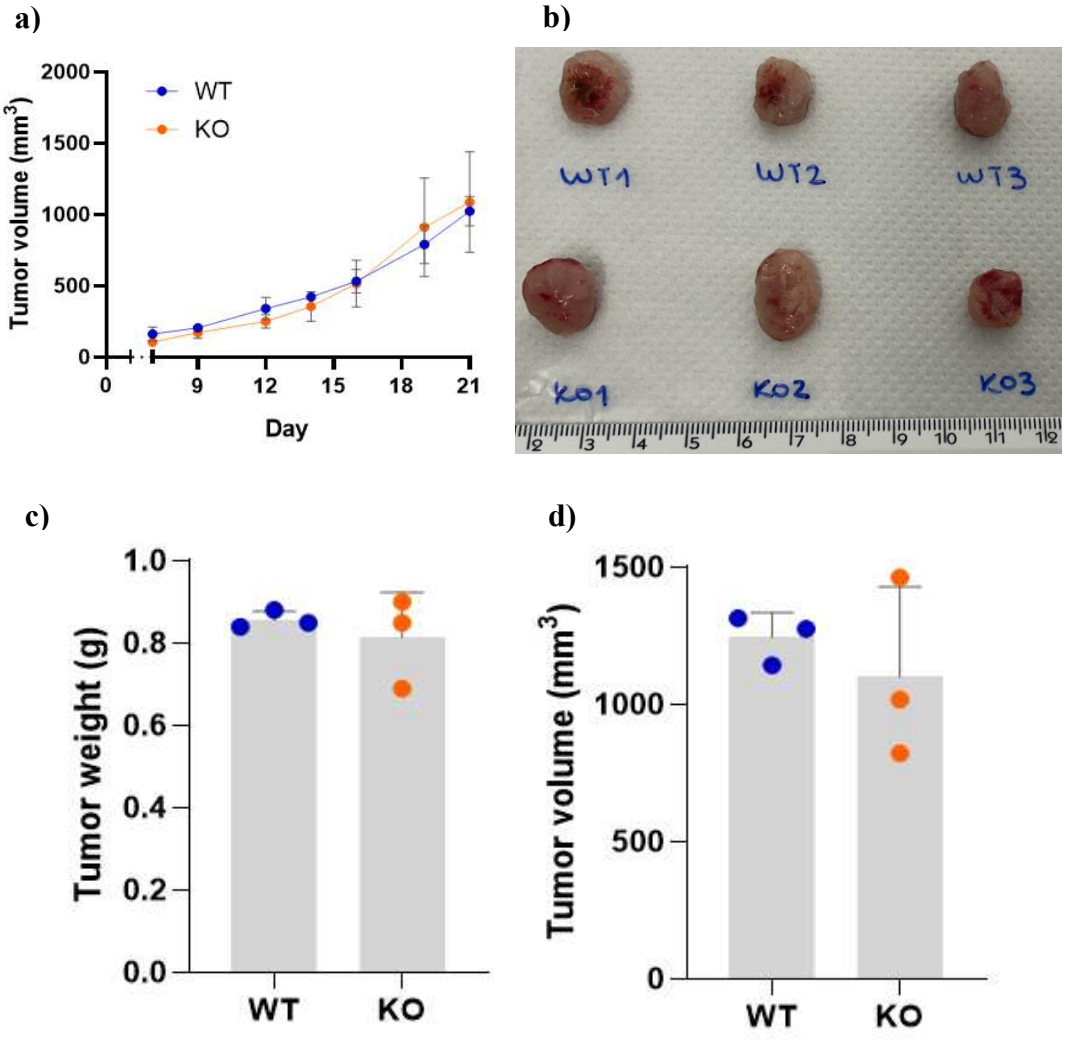

Figure 1. Evaluation of subcutaneous tumor growth in CD300e KO and WT mice. a) Tumor volume measured over the observation period using the formula ($L \times l^2$)/2. Statistical significance was assessed using a 2-way ANOVA test and no statistically significant difference was observed between the two groups ($p > 0.05$). b) Tumors isolated from mice at sacrifice. c) Final tumor weight in grams. The results are presented as mean values with standard error of the mean (SEM), and normality was assessed using the Shapiro-Wilk test. Statistical significance was assessed using an unpaired Student's t-test. Tumour weights were similar among WT mice and most KO mice, with one KO mouse showing a slightly smaller tumour. No statistically significant difference was observed between the groups ($p > 0.05$). d) Final tumor volume in mm^3, calculated as H x W x L. The results are presented as mean values with SEM, and normality was assessed using the Shapiro-Wilk test. Statistical significance was assessed using an unpaired Student's t-test. Tumor volumes were similar among WT and KO mice, with one KO mice showing a slightly smaller volume compared to the rest. No statistically significant difference was observed between the groups ($p > 0.05$).

Weight measurement (Fig.1c) reveals that WT mice had slightly heavier tumours compared to KO mice. All three WT mice had tumours within a weight range of approximately 0.8 to 0.9 grams. In contrast, two of the KO mice followed this trend while the third one had a tumour weighting a little less than 0.7 grams. Despite this difference, statistical analysis using an unpair t-test showed no significant difference in tumor weight between WT and KO groups.

Tumor volume at sacrifice (Fig.1d) appeared similar between the two groups. All three WT mice had consistent tumor volumes, while one KO mice showed a deviation from the group trend. Statistical analysis using an unpaired t-test confirmed that the difference in tumor volumes between groups was not statistically significant.

3.2. Phagocytic Activity Analysis

Phagocytic activity of isolated TAMs was evaluated at confocal microscope (Fig. 2a, b). Phagocytic index (Fig. 2e) was determined by multiplying the percentage of phagocytic macrophages (Fig. 2d) per the mean number of phagocyted beads per macrophage (Fig. 2c). As it can be observed from Fig. 2c, there is a slightly higher number of phagocyted beads in KO TAMs. However, the difference between WT and KO groups is minimal, suggesting that individual macrophages from both groups engulf a similar number of particles. In contrast, the percentage of phagocytic macrophages (Fig. 2d) reveals statistically significant differences, with a higher number of KO TAMs being phagocytic. As a result, the phagocytic index (Fig. 2e) is significantly higher in the KO group compared to WT, suggesting that macrophages from KO mice are more efficient at phagocytosis. These findings suggest that the absence of CD300e improves the ability of TAMs to perform phagocytosis; without CD300e, macrophages are better at engulfing particles and might be better able to destroy cancer cells.

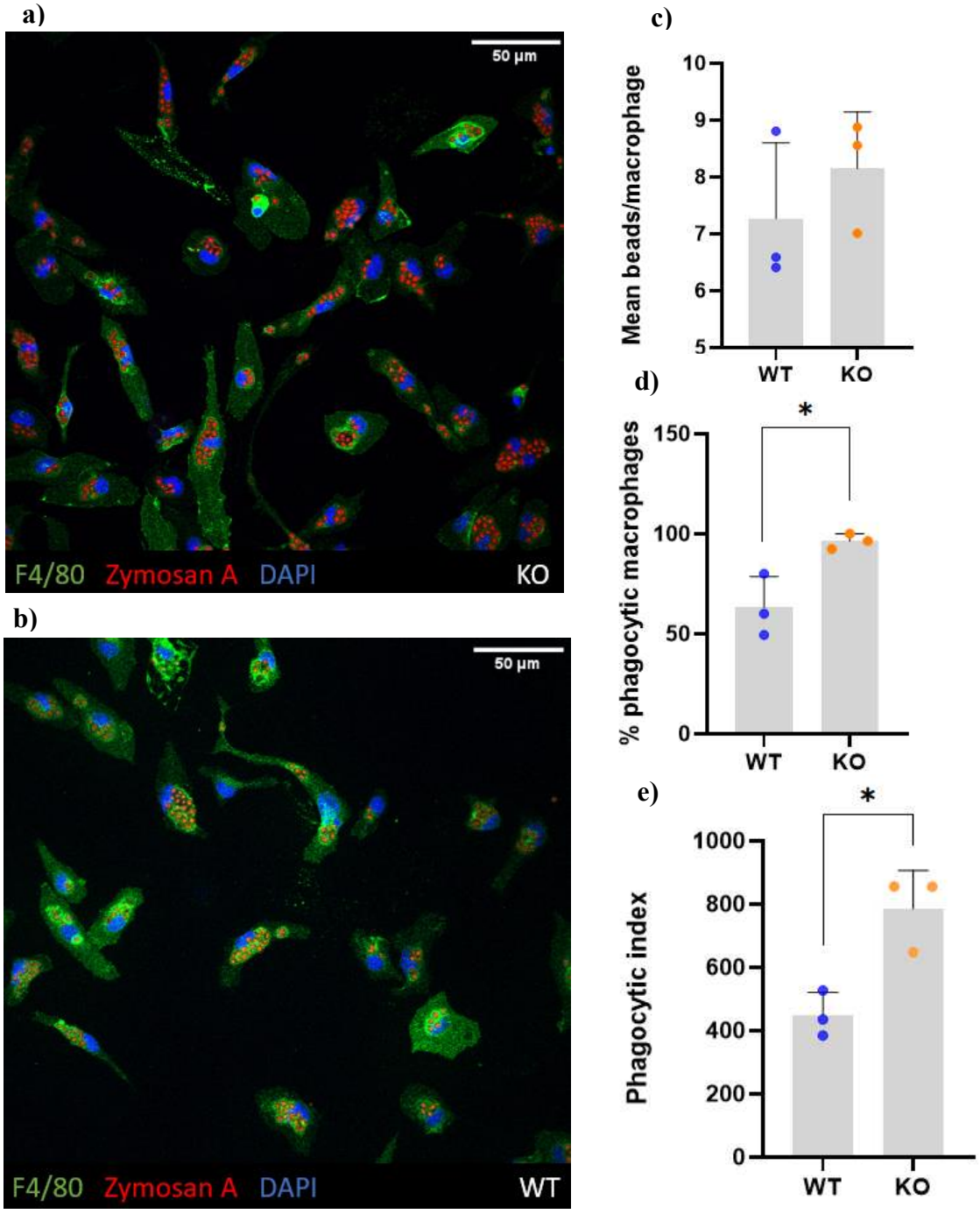

Figure 2. Immunofluorescence analysis and quantification of macrophage phagocytic activity in CD300e KO and WT TAMs. (**a, b**) Representative immunofluorescence images of macrophages from KO (**a**) and WT (**b**) mice. Macrophages were labelled with anti-F4/80 antibody (green), nuclei were stained with DAPI (blue), and Texas Red Zymosan A bioparticles (594 nm, red) were used. Images represent maximum intensity projections and were processed and analysed using Fiji (ImageJ). Scale bar = 50 µm. (**c**) Mean number of beads internalized per macrophage. The results are presented as mean values with SEM, and normality was

assessed using the Shapiro-Wilk test. Statistical significance was assessed using an unpaired Student's t-test. No statistically significant difference was observed between the groups ($p > 0.05$). (**d**) Percentage of phagocytic macrophages. The results are presented as mean values with SEM, and normality was assessed using the Shapiro-Wilk test. Statistical significance was assessed using an unpaired Student's t-test. Statistically significant difference was observed between the two groups ($p < 0.05$, indicated by *) (**e**) Phagocytic index, calculated by multiplying the percentage of phagocytic macrophages by the mean number of beads per macrophage. The results are presented as mean values with SEM, and normality was assessed using the Shapiro-Wilk test. Statistical significance was assessed using an unpaired Student's t-test. Statistically significant difference was observed between the two groups ($p < 0.05$).

4. CONCLUSION

The aim of this study was to investigate the role of CD300e in CRC and whether this receptor can impact TAMs. Using a subcutaneous tumor model, WT and CD300e KO mice were injected with MC38 colon cancer cells and monitored over a 21-day period. At the end of the experiment, tumor volume and weight were measured. No statistically significant differences were found between the two groups, which contrasts with previous studies that observed delayed tumor growth in CD300e KO mice. This difference may be due to the small sample size used in this experiment.

Despite the similarities in tumor sizes, differences in TAM function were observed. A phagocytosis assay revealed that a significantly higher percentage of TAMs from CD300e KO mice were actively engaging in phagocytosis. Although individual macrophages from both groups phagocytosed a similar number of particles, the increased percentage of phagocytic TAMs in the KO group led to a higher phagocytic index. This suggests that CD300e reduces the number of active, phagocytic macrophages within the TME.

These findings are consistent with earlier research showing that CD300e impairs antigen presentation and reduces T cell activation. Consequently, knocking out CD300e improves antigen presentation, leading to enhanced T cell activation and an anti-tumor response. Moreover, our results indicate that CD300e may limit the ability of macrophages to perform phagocytosis, a function crucial for destroying tumors, and to present antigens. Since CD300e pushes TAMs towards a tumor-promoting profile, targeting this receptor could reprogram TAMs toward an anti-tumor, immunostimulatory profile.

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