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Memory T-cell dynamics following immunization with a polyvalent salmonellosis vaccine in farm animals

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Abstract

The topic of studying the pool of memory T-cells during immunization with vaccines is relatively understudied, but this is no less important. The research aims to investigate the body's accumulation and storage time of immune memory cells. The study used the following methods: gradient centrifugation method, flow cytofluorometry method, direct immunofluorescence reaction, four color compositions method, cluster analysis, and methods of calculating the arithmetic mean and its standard error. The study examined rabbits at 1.5 months of age. The study of quantitative changes in memory T-cells and their phenotypes showed an increase in central and effector CD4+ and CD8+ memory T-cells. Also, quantitative differences in memory cell phenotypes were observed in primary immunized animals compared to booster immunization. Most frequently, specific CD4+ T-cells, which produce cytokines, could be seen in the lymphocytes of the own lamina of the muscularis ileum and jejunum. A significant difference in the notional number of cytokine-producing characteristics between the group not subjected to the experiment and the immunized animals was dominated by CD4+ T-cells in the intestine and lymph nodes. This study showed essential factors in the development of immune cell memory and will be useful both for farmers and for the development of the topic of memory in immunization.

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Introduction

A study of the memory T-cell pool during immunization with a polyvalent vaccine against Salmonellosis in farm animals can answer essential questions about the functioning of many immune system components. Due to its high zoonotic potential, the pathogen *Salmonella enterica* serotype Typhimurium is a dangerous microorganism for animals and humans. The lack of awareness on this subject is unacceptable and needs to be investigated as soon as possible. At the same time, these studies are costly and require highly qualified staff. That is why studying similar and related works by other authors is important. Investigating the bacteria, Ryskeldinova *et al.* (1), Minervina *et al.* (2), and Peeters *et al.* (3) found that *Salmonella Typhimurium* is an intracellular Gram-negative facultative bacterium. It is part of the family *Enterobacteriaceae* and can infect a wide range of hosts. *Salmonella Typhimurium*, as part of the non-brucellosis serovars of *Salmonella*, is a frequent cause of gastroenteritis in humans. This is due to its zoonotic properties; it also poses a constant risk to food safety. Besides eggs and their derivatives, rabbits and rabbit meat are also causes of *Salmonella Typhimurium* in humans, using the food chain. Mahmudov and Sattori (4) and Paprckova *et al.* (5) pointed out in their research that immunological memory has now been the basis of adaptive immunity. It is also recognized that T-lymphocyte populations are subdivided into naive T-cells and memory T-cells (Tm), in which CD4+ and CD8+ phenotypes are present. In animals, as in humans, the recognized marker for naïve and memory T-cell differentiation is the transmembrane CD45, which is present in all blood cells.

Human T-lymphocytes are divided into four varieties. These varieties result from splicing exons of the same gene. In turn, Radbruch *et al.* (6) suggest that naive T-lymphocytes have CD45RA+, CD45RO- phenotypes, and memory cells have CD45RA-, CD45RO+ phenotypes. Depending on L-selectin (CD62L) composition and the chemokine receptor CCR7, memory cells are divided into the CCR7+, CD62L hi phenotype, and the CCR7-, CD62L lo phenotype. At the same time, Tm effectors are divided into typical 45RA-, CCR7-, and CD62L lo phenotypes and differentiated CD45RA+, CCR7-, and CD62L lo phenotypes. In a similar study, Tulegenov (7) and Sultanov *et al.* (8) pointed out that the sources studied indicate that when an antigen is introduced, both during infection and as a result of immunization, only antigen-recognizing cells, which constitute a small number of the entire lymphocyte diversity, are activated in response. Only a small number turn into memory cells, while most remaining cells, which have receptors for other antigens, remain dormant. A study by Abdrakhmanov *et al.* (9) and Lanfermeijer *et al.* (10) noted that the transformation of T-cell subpopulations can also be detected using non-specific markers. The authors also noted that memory cells localize most of the time in organs and tissues that have a barrier function because, unlike the vast majority of naïve (central) phenotypes, this is where they localize, migrating into the bloodstream for a short time. Of course, this fact cannot serve as an indicator of immunity strength in its entirety. Following Zakharov *et al.* (11) and Müllebner *et al.* (12), the memory T-cells are most important for creating long-term immunity. Depending on the number of responsively generated effector T-cells, the number of memory T-cells is regulated, while an additional set of factors also has the potential to increase the quality and efficiency of memory cell recruitment. Cell proliferation and cytokine-mediated regulation of survival have a crucial influence on Tm-cell pool homeostasis. Creating defenses against pathogens that have emerged because of unique host relationships, different host entry and replication points, and colonization in specific T-cells requires adaptable and diverse immune system responses. This adaptability and diversity, critical for effective host immune surveillance, are facilitated by heterogeneous CD8 memory T-cells. The time factor after infection and novel antigens lead to further diversity in the shape of the CD8 memory T-cell pool. This has implications for CD8 T-cell-mediated protection against reinfection (13-15). An examination of the works of other authors makes clear the incompleteness of the study of this topic and the possible ways in which it could be pursued.

The study aimed to investigate the accumulation, storage time, and phenotypic changes of immune memory cells in the body after immunization of farm animals with a multivalent salmonella vaccine. In particular, the following tasks were set to [1] Determine changes in the number of central memory T-lymphocytes (TCM, CD45RA-, CCR7+) and effector memory T-lymphocytes (TEM, CD45RA-, CCR7-) in response to immunization with Salmonella antigen, [2] To investigate changes in the phenotypes of T-lymphocyte subpopulations, including naive (CD45RA+, CD62L+), effector (CD45RA+, CD62L-), central memory (CD45RO+, CD62L+), and effector memory (CD45RO+, CD62L-) cells after immunization, [3] To study the dynamics of memory T-lymphocyte subpopulations over time, including after primary and booster immunizations, [4] To investigate the numerical composition and production of cytokines (IFN-γ, TNF-α, IL-17A) by salmonella-specific CD4+ T cells in different tissues (intestine, lymph nodes, tonsils) after immunization, and [5] To investigate the formation of CD45RA+ memory effector T cells as an indicator of repeated antigen exposure and secondary immune response.

Materials and methods

The experiment was conducted on groups of rabbits in rabbit house No. 1 of farm No. 4 in the Karaganda region of the Republic of Kazakhstan. The experimental group of animals studied consisted of 20 rabbits, 1.5 months old, weighing 2 to 2.7 kg. The animals had not been previously vaccinated and were not ill. All animals were vaccinated with a polyvalent vaccine against Salmonellosis from farm animals containing vaccine strains of *Salmonella*. A vaccine manufactured by Cid Lines company was used in the study. The vaccination scheme involves primary immunization of rabbits at 1.5 months of age, followed by a booster immunization for a subset of rabbits six months after the primary dose, utilizing an intramuscular injection of a polyvalent vaccine against Salmonellosis containing vaccine strains of Salmonella. The study evaluates various aspects of the immune response to Salmonella antigen immunization. This includes assessing changes in the number and phenotypes of memory T cell subpopulations, investigating the dynamics of memory T cell numbers over time post-immunization, examining the composition and cytokine production of Salmonella-specific CD4+ T cells in different tissues, and studying the formation of effector memory T cells as an indicator of antigen re-encounter and secondary immune response after booster immunization.

Blood samples from laboratory animals were taken according to the following scheme: one day before antigen administration and 7 and 14 days after immunization. The animals were monitored clinically daily. Given changes in behavior, rectal temperature, vomiting, and diarrhea, observations were evaluated and recorded using a point system. At the same time, the body weight of all animals was recorded every week. After euthanasia, using visual inspection, the internal organs of each rabbit were assessed for the formation of pathological changes. Blood samples were taken from the ear vein. Serum was obtained by centrifugation of blood samples for 10 minutes at 1800 rpm at room temperature. Membrane CD markers were

determined by flow cytofluorometry and direct immunofluorescence reactions. For this purpose, BD Biosciences reagents and technologies were used: the FACSCalibur cytometer and CellQuest data processing software. The lymphoid region was isolated by CD45/CD14 staining using light scatter diagrams with control of the purity of lymphocyte isolation by CD45 expression (95–98% CD45+ cells in the region). Four color compositions of the following surface markers were used to determine lymphocyte subpopulations: CD3, CD4, CD8, CD45RA, and CD45R0.

To release the lymphocytes into their plates from the ileum and jejunum, the previously opened intestine was washed with sterile phosphate-salt buffer and then cut into small pieces. Afterward, the tissue was incubated for 50 min at 38℃ on a shaker in HBSS to release intraepithelial lymphocytes. After this incubation, the supernatants were removed, and the remaining tissue was placed in a cell culture medium; the cell sludge was filtered through a cell sieve and resuspended. The interphase cells were selected and washed twice with PBS and once with culture medium. The cells were resuspended in the nutrient substrate with amikacin sulfate and 15% fetal calf serum. The amount of each cell preparation was determined on a BC-5150 hematology analyzer.

Antigens for in vitro stimulation of lymphocytes were prepared as follows. The vaccine strain was grown in two previous cultures in the internal medium at 38.2℃ at approximately 160 rpm on a mixer. This was followed by centrifugation of the sample for 10 minutes at 7500 rpm. The coagulate is again formed into a suspension in sodium thiosulphate. The samples, which generate colonies, are isolated, blocking the activity of the concentrate by heating in the thermostat for 75 min at 55℃. The antigen was then separated into aliquots. It was then interrupted at -70℃ until the use time (16). Blood T-lymphocyte counts, and counts of different phenotypes of T-cell subpopulations were studied. The blood counts, including the T lymphocyte counts, were evaluated using an automated hematology analyzer.

Ethical approval

A study was approved by the Ethics Commission of the Kazakh National Agrarian Research University on November 15, 2023, No. 153-A.

Statistical analysis

Cluster analysis was organized according to items: score (least-squares contrasts between control and studied rabbits), SP (standard errors of estimated contrasts), t-test (t-test statistics), p-values (nominal p-values), correction of p-values (p-values were corrected for multiple testing using the false discovery rate). Appropriate algorithms were exposed. Cluster analysis was then performed for interleukin 17-A (IL-17A), tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ) activated by Salmonella bacteria, creating CD4+ cells. Salmonella primarily breaches the intestinal barrier and infects the gastrointestinal tract. To investigate the point response of immune system components, such as T-lymphocytes, at a given location of host-pathogen interaction, the authors first performed a cluster analysis for two intestinal compartments (the ileum and jejunum). The research results were processed by calculating the arithmetic mean and its standard error (M±SE). The significance of differences between the mean values was assessed by the student's t-test (t). Differences were considered significant at $p<0.05$. All sampling followed the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (17) and the Universal Declaration on Animal Welfare (18).

Results

After the animals were brought into specially prepared cages, an increase in rectal temperature was observed in the rabbit groups. It was kept around 39.4-40.2℃, increasing by 0.27℃ in the unexperimented group and 0.16℃ in the studied group, but only for one day. This phenomenon was most likely due to the nervousness of the change of location and habituation to the new conditions. At the end of this process, the temperature of all animals was in the normal range until the end of the experiment. The study's first objective was to determine changes in the number of CD45RA and CD45RO memory cells in response to immunization. A significantly higher number of Tm was detected. There was an increase in the number of double-positive cells, which was maintained even on day seven after vaccination ($p<0.01$), a trend that continued even 14 days after vaccination. The results are presented in table 1.

Table 1: Number of naive T-cells and memory cells in rabbit blood

Groups	Before vaccine	After 7 days	After 14 days
45RA+	71.25±1.18	79.28±1.47	76.81±1.24
45RO+	7.85±1.51	9.81±1.32	9.21±1.34
45RA+, 45RO+	0.53±0.11	0.68±0.38	0.82±0.22

The next stage of research was to investigate changes in T-lymphocyte phenotypes. It was shown that the number of subpopulations of naive (central) T-lymphocytes (CD45RA+, CD62L+) increased. Activated populations produced during primary immunity (CD45RA+, CD62L-), central (CD45R0+, CD62L+) and memory cells (CD45RO+, CD62L-) were also identified. It was found that most naïve cells and memory cells had CD4+ markers, but CD8+ memory cells could also be identified. The results are presented in table 2.

Table 2: Rabbit blood T-cell subpopulations

CD4+				CD8+			
CD45RO+		CD45RA+		CD45RO+		CD45RA+	
CD62L+	CD62L-	CD62L+	CD62L-	CD62L+	CD62L-	CD62L+	CD62L-
4.98±0.39	0.81±0.18	28.14±0.94	1.56±0.17	2.12±0.12	0.62±0.13	13.11±0.23	1.39±0.58

In the first part of the experiment, all rabbits were initially immunized at 1.5 months. Two months after vaccination, one group of rabbits was examined to evaluate the effectiveness of immunization. This evaluation aimed to analyze markers of immune response, such as the presence of specific T cells and antibodies, indicating successful immunization. The second group of rabbits received revaccination before six months after the first vaccination. This was done to study the effects of revaccination on immune system memory and overall performance after a shorter period than usual.

This part of the study was critical in determining how quickly the immune memory responds to repeated exposure to an antigen, and it helped to understand the optimal time for revaccination in the vaccination schedule. Both settings study the dynamics of memory T cells after vaccination and how re-immunization can enhance or maintain the immune response. The methods used, such as blood sampling and monitoring of clinical signs, allowed for detailed observations of immune function over time. The results presented in figures 1 and 2 detail changes in T-cell populations and other immunologic markers that determine the success of the vaccination process.

With age, there are changes in the numbers of both types of memory cells, but more pronounced in the CD4+ population than in CD8+ cells. Measurement of the first principal component analysis showed a clear separation of the data points obtained from the intestinal tissue into a pair of groups: the material obtained from the vaccinated animals under study was divided into clusters according to given criteria together, on the one hand. At the same time, each of the materials obtained from the control and test animals cultured only in the medium formed a cluster. Thus, the measurement of the first principal component analysis resulted from treating the animals (controls against infection and immunization) and activating specific parts of the material (medium against Salmonellosis antigen).

In the original calculation, approximately 76% of variants were in each of the two bowel divisions. The following calculation yielded 7.9% and 9.5% of variants in the ileum and rectum, explained by the cytokine production variation. IFN-γ/IFN-α and IFN-γ co-producing cells co-segmented in the upstream sector, while IL-17A-interacting phenotypes co-segmented in a different route. To establish the apparent differences between the control and study groups, p-values for all organs and phenotypes were calculated, and corrections were made where necessary, considering multiple controls (p-values of 0.1 or less were indicative). Significant differences between the control and study groups were found for 5 7 phenotypes in each bowel region (Table 3).

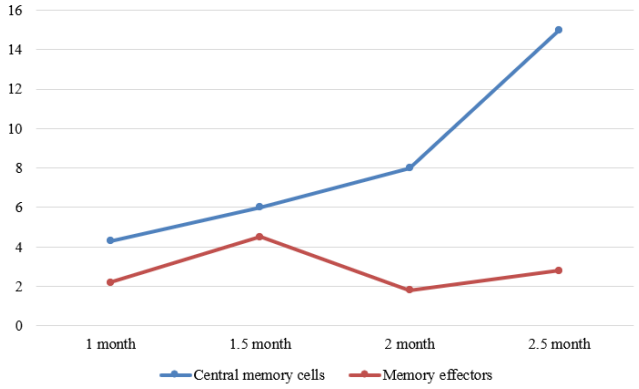

Figure 1: Age-related changes in the number of CD4+ T-cells (% of T-lymphocyte count).

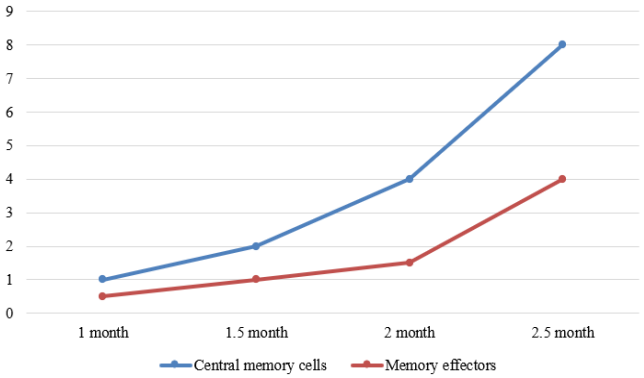

Figure 2: Age-related changes in the number of CD8+ T-cells (% of T-lymphocyte count).

Phenotypes are not significantly different between groups: IFN-γ-producing CD4+ T-cells in the small intestine, TNF-α/IL-17A co-producing CD4+ T-cells in the ileum, and IL-17A-producing CD4+ T-cells in each intestinal tissue. Given the available population of organs of the lymphatic system, clustering according to the criteria in each intestinal lymph node was equivalent to the situation observed in the ileum and jejunum, both for principal component analysis and mapping. In the mesenteric lymph nodes presented, Salmonella-activated samples of the studied group clustered far from control and medium-only

samples, the former accounting for approximately 70% of the variation. Notably, CD4+ T-cells producing single IFN-γ clustered in isolation compared to the other features when passing through principal component analysis.

As such, differences in the relative numbers of individual IFN-γ producers among the groups in the two lymph nodes studied achieved little significance. Compared to the intestinal studies, feature sets including IL-17A equally produced closely spliced clusters in the two lymph nodes. P values for the two lymph nodes showed large discrepancies between the group not subjected to the experiment and samples from animals in the study group for TNF-α/IL-17A co-producing CD4+ memory effector cells, IFN-γ/IFN-α co-producing cells, and TNF-α co-producing cells. Also, in addition to the above, significant inconsistencies were acquired for IFN-γ/IL-17A co-production and IFN-γ/IFN-α/IL-17A triple production by CD4+ memory effector cells in the iliac-obstructed lymph nodes. However, in the amygdala, the distribution of the vaccine-activated parts of the test material from animals not subjected to the experiment and the medium-only material had worse clarity. Accordingly, no significant differences could be found in the number of cytokine-producing phenotypes between the groups in the amygdala (Table 4). The next step was to study the cell number dynamics in Tm-cell subpopulations in response to immunization (Figures 3 and 4).

Table 3: Calculation of Salmonella-specific CD4+ T-cell cytokine responses in the jejunum and ileum

Material	Phenotype	Mark	SE	t-test	p-values	Correction of p-values
Small intestine	IFN-	-1.895611	0.968731	-2.166841	0.058745	0.013586
	FNO-α+	-0.356148	0.236589	-3.254138	0.003697	0.045723 (p<0.01)
	IL-17A+	-0.189756	0.143257	-1.246653	0.256321	0.369941 1
	IFN-γ+, FNO-α+	-0.719342	0.169872	-2.741336	0.264513	0.054334
	IFN-γ+, IL-17A+	-0.098762	0.281136	-2.003451	0.074625	0.078332
	FNO-α+, IL-17A+	-0.091354	0.039222	-2.166223	0.025866	0.098659 (p<0.05)
	IFN-γ+, FNO-α+, IL-17A+	-0.126587	0.041783	-3.015952	0.005852	0.047532 (p<0.05)
Ileum	IFN-	-1.816973	0.640268	-2.595621	0.039636	0.051231 (p<0.05)
	FNO-α+	-0.423861	0.133084	-3.000564	0.002053	0.044983 (p<0.01)
	IL-17A+	-0.038956	0.089964	-0.345621	0.748884	0.777665
	IFN-γ+, FNO-α+	-0.739514	0.241633	-2.741268	0.015984	0.036123 (p<0.05)
	IFN-γ+, IL-17A+	0.127836	0.059634	-2.911134	0.007968	0.071224 (p<0.01)
	FNO-α+, IL-17A+	0.068359	0.045211	-2.035691	0.063114	0.112566
	IFN-γ+, FNO-α+, IL-17A+	0.153782	0.489635	-3.112633	0.006123	0.039786 (p<0.05)

Note: Mark – contrasts between least-squares values of control and test rabbits; SE – standard errors of estimated contrasts; t-test – t-test statistics; p-values – nominal p-values; correction of p-values: p-values were corrected for multiple testing using the false discovery rate.

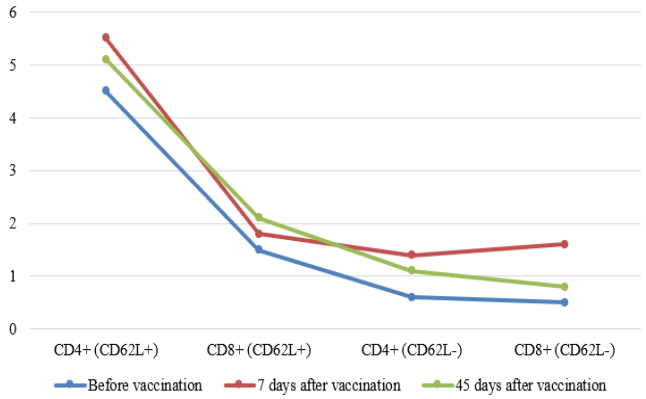

Figure 3: Change in the number of memory T-cells after the first salmonellosis vaccination (% of the number of T-lymphocytes).

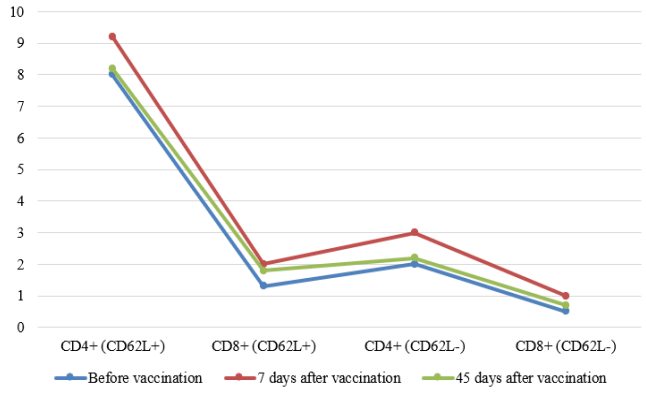

Figure 4: Change in the number of memory T-cells after the booster salmonellosis vaccination (% of the number of T-lymphocytes).

Table 4: Calculation of Salmonella-specific CD4+ T-cell cytokine responses in the jejunal lymph node (TLU), iliac-oesophageal lymph node (ILN) and tonsils

Material	Phenotype	Mark	SE	t-test	p-values	Correction of p-values
TLU	IFN-	0.068422	0.074412	0.097521	0.412355	0.362264
	FNO-α+	-0.135542	0.043255	-2.70125	0.012544	0.040132
	IL-17A+	-0.007935	0.010532	-0.761241	0.478211	0.441804
	IFN-γ+, FNO-α+	-0.047122	0.003456	-2.536621	0.021146	0.060511
	IFN-γ+, IL-17A+	-0.003872	0.005731	-1.522147	0.239874	0.214265
	FNO-α+, IL-17A+	-0.013655	0.007961	-2.236122	0.034406	0.073401
	IFN-γ+, FNO-α+, IL-17A+	-0.017751	0.008161	-2.041412	0.040473	0.111538
POLU	IFN-	0.004602	0.014235	0.263847	0.600545	0.647057
	FNO-α+	-0.046545	0.021787	-1.647857	0.011128	0.040132
	IL-17A+	-0.001701	0.001611	-1.050504	0.287102	0.038945
	IFN-γ+, FNO-α+	-0.012436	00.3742	-2.681703	0.011048	0.040132
	IFN-γ+, IL-17A+	-0.003245	0.000443	-2.574803	0.014338	0.037062
	FNO-α+, IL-17A+	-0.004364	0.001327	-3.093186	0.003307	0.023421
	IFN-γ+, FNO-α+, IL-17A+	-0.004454	0.001243	-3.886834	0.000604	0.02777
Tonsils	IFN-	0.0325611	0.021656	1.537733	0.103245	0.172531
	FNO-α+	-0.123654	0.055446	-2,.31201	0.004652	0.125214
	IL-17A+	0.001422	0.004221	0.234156	0.752651	0.811525
	IFN-γ+, FNO-α+	-0.135221	0.006875	-1.521341	0.085615	0.154744
	IFN-γ+, IL-17A+	-0.002448	0.002451	-1.644218	0.412534	0.143355
	FNO-α+, IL-17A+	-0.00313	0.003617	-1.345211	0.851244	0.395551
	IFN-γ+, FNO-α+, IL-17A+	-0.003138	0.006778	-1.324563	0.132256	0.187442

Note: Mark – contrasts between least-squares values of control and test rabbits; SE – standard errors of estimated contrasts; t-test – t-test statistics; p-values – nominal p-values; correction of p-values: p-values were corrected for multiple testing using the false discovery rate.

In animals immunized for the first time, there was no change in the number of values in the populations on day 7. After immunization, CD4+ and CD8+ effector memory cells were significantly increased after 1.5 months ($p<0.05$). On repeat immunization after six months, the laboratory animals recorded increased CD4+ and CD8+ subpopulations in the central and effector cell populations on day 7. After 45 days, a decrease in both subpopulations was recorded. However, as expected, the numbers were higher than the baseline numbers observed before the booster immunization. In a study of the effector subpopulations capable of forming CD45RA on themselves, an increase was observed one week after the initial vaccination. The authors consider this to be an indicator of an initial immune response. The high level is maintained 45 days after the primary immunization. At re-immunization, a rapid secondary immune response with the accumulation of immune memory cells, which decreased but were higher than the initial level, was observed 45 days after the re-introduction of the antigen (Figures 5 and 6).

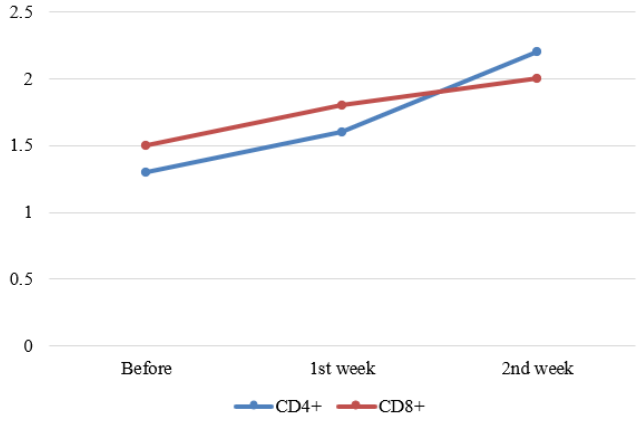

Figure 5: Change in the number of CD45RA+ effectors after administration of Salmonella vaccine in the first group (% of T-lymphocytes).

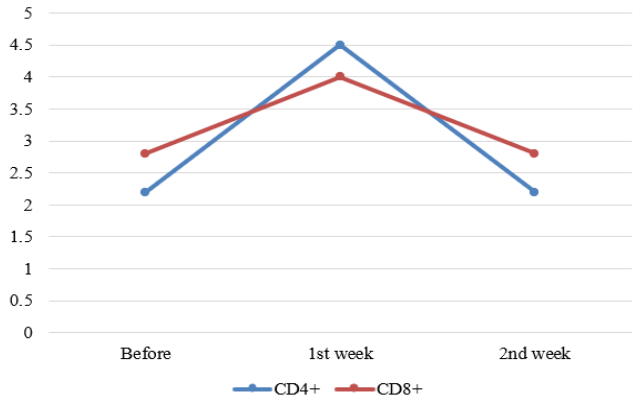

Figure 6: Change in the number of CD 5RA+ effectors after administration of Salmonella vaccine (% of T-lymphocytes).

Discussion

The research showed that changes occur in memory T cells when immunized. It is, therefore, important to consider other studies and relate them to the experiment above (19). The increase in central memory (TCM, CD45RA-, CCR7+) and effector memory (TEM, CD45RA-, CCR7-) T cell populations following Salmonella vaccination, as observed in this study, aligns with the basic definition and characteristics of memory T cells described by Booth *et al.* (20), Esser *et al.* (21). Smith *et al.* (22). Memory T cells are antigen-experienced cells that persist for an extended period after the initial antigen encounter. The ability of the memory T cells generated by Salmonella vaccination to rapidly proliferate, perform effector functions like cytokine secretion, and traffic to peripheral tissues upon antigen re-exposure, as demonstrated by the increase in effector memory phenotypes (CD45RO+, CD62L-) after booster immunization, supports the qualitative and quantitative differences between memory and naive T cells highlighted by these authors. The maintenance of elevated memory T cell levels even after 45 days post-booster, although declining from the peak, agrees with the long-term persistence and greater retention rate of memory cells compared to short-lived effector cells, as described in these studies. The distinct kinetic patterns observed for primary vs. secondary (booster) immunization, with a more rapid memory response upon booster, likely result from the pre-existing pool of antigen-experienced memory cells formed after primary immunization, consistent with the explanations provided. The detection of cytokine-producing CD4+ T cells, especially in mucosal sites like the intestine where Salmonella infections occur, underscores the ability of memory T cells to mount rapid effector responses like cytokine secretion upon antigen re-encounter, a key trait distinguishing them from naive T cells.

In turn, Chang and Radbruch (23), as well as Krishnamurty and Turley (24), found that living organisms fall victim to the same infectious agents throughout their lives in most cases. Lists of primary booster immunization rules and regulations designed to increase memory cell numbers lead to repeated exposure of CD8 memory T-cells to antigens. Initial studies using adaptive translocation of purified genetically modified TCR memory cells or positive tetrameric cells with a familiar final antigen effect reported that the antigen booster effect caused a reduction in the proportion of translocating effector CD8 memory cells converting CD127, CD62L, and CD27 and an increase in the proportion of KLRG1 and GranzymeB-converting cells. Equally, the phenotype of secondary CD8 memory T-cells is affected by the complex inflammatory process triggered by vaccinations or infections. Invariable antigen encounters similarly induce increased changes in the transcriptome numbering of memory CD8 T-cells (25-30).

Memory-effector genes are not enhanced by antigen encounters (31-33). This suggests that accessory antigen encounters cause an increase in memory T-cell numbers (34). Differences in the structure and function of CD8 memory effector cells under the added influence of antigen resulted in various ways to provide countermeasures against reinfection (35-37). However, CD8 effector memory cells influenced by several antigens show enhanced resistance against LCMV-Armstrong, *Listeria monocytogenes*, and smallpox virus and reduced resistance against reinfection by LCMV clone 13 and MHV (38-40). Nevertheless, secondary homologous feeding preserves total resistance and increases the phenotypic and functional complexity of the CD8 memory effector cell pool (41). At the same time, further heterologous infection induces the production of a pool of antigen-specific CD8 memory effector cells with a phenotypic profile expressing major metabolically adapted memory effector cells, which rapidly proliferate upon repeated infection and create resistance to LCMV clone 13. These indicators are similar to those given above and confirm the validity of the authors' findings.

Our results showed that the frequency of Salmonella-specific, cytokine-producing CD4+ T cells was highest in the intestinal lamina propria and ileum compared to other organs like lymph nodes and tonsils. This observation is similar to the findings of Campos *et al.* (25), Martin, and Badovinac (26) in pigs, where *Salmonella Typhimurium* infection was mainly confined to the intestine, with limited systemic spread observed. The higher frequency of Salmonella-specific CD4+ T cell responses in the intestine is likely because this is the primary site of Salmonella entry and infection in the host. Salmonella must breach the intestinal epithelial barrier to establish infection as an intracellular pathogen. Therefore, the intestinal mucosa is a crucial inductive site for generating Salmonella-specific CD4+ T cell responses to control the local infection.

A significant number of characterizations sets with large differences among the examined and control groups of pigs were found in the examined secretory lymph nodes of the

intestine, with seven phenotypes predicted to produce cytokines, almost all induced in the intestinal segment. This is consistent with studies in mice and humans, where endogenous T-cell lamellae have been identified as an integral component of the response to *Salmonella Typhimurium* and *Salmonella Typhi*. The number of numerous Salmonella cytokine-specific CD4+ T-cells in plaques located in the intestines of the animals studied in this study suggests that they may also play an important role in the mucosal immune response to Salmonella Typhimurium infection in pigs. These observations are confirmed in the study above, although the animal species are quite different. The findings showed that the data found and obtained coincided with other authors' descriptions. The discussion on this issue provided an opportunity to bring in more details, thereby increasing the scope of the topic and allowing for a better understanding of it.

Conclusions

This study investigated the changes in the memory T cell pool following immunization with a polyvalent Salmonella vaccine in rabbits. The results demonstrated the accumulation of central memory (CD45RA-, CCR7+) and effector memory (CD45RA-, CCR7-), CD4+, and CD8+ T cells after vaccination. A key finding was the quantitative differences observed in the memory T cell phenotypes between primary and booster-immunized animals. Primary immunization led to a delayed accumulation of effector memory cells, while booster vaccination induced a more rapid effector memory response. This likely results from the pre-existing pool of antigen-experienced memory cells formed after the initial immunization, allowing for an accelerated recall response upon antigen re-exposure during the booster.

Interestingly, the highest frequencies of Salmonella-specific, cytokine-producing CD4+ T cells were detected in the intestinal lamina propria and ileum compared to other tissues like lymph nodes and tonsils. This localized mucosal response is expected given that the intestine is the primary site of Salmonella entry and infection. Detailed analysis showed significant increases in IFN-γ, TNF-α, and IL-17A-producing CD4+ T cells, especially co-producing phenotypes, in immunized animals compared to controls in these mucosal and lymphoid tissues.

A notable observation was the rapid increase in effector memory T cells expressing CD45RA (CD45RA+, CCR7-) after booster immunization. These cells may serve as an indicator of antigen re-encounter and initiation of the secondary immune response involving rapid expansion of the antigen-experienced effector memory pool. This study provided insights into the generation, kinetics, phenotypes, and tissue distribution patterns of the central and effector memory T cell pools induced by Salmonella vaccination in the rabbit model. These findings enhance our understanding of the memory T cell response against this mucosal pathogen.

Conflict of interest

There is no conflict of interest.

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ديناميكية خلايا الذاكرة التائية بعد التحصين بلقاح داء السالمونيلا متعدد التكافؤ في حيوانات المزرعة

سابباركان ديموفيتش زانابابيف و بيرزان كاديروفيتش بياشيف

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الخلاصة

إن موضوع دراسة مجموعة خلايا الذاكرة التائية أثناء التحصين باللقاحات لم تتم دراسته بشكل كافٍ نسبيا. يهدف البحث إلى دراسة تراكم وزمن تخزين خلايا الذاكرة المناعية. استخدمت الدراسة الطرق التالية: طريقة الطرد المركزي المتدرج، طريقة قياس التدفق الخلوي، تفاعل المناعي المباشر، طريقة تركيبات الألوان الأربعة، التحليل العنقودي، وطرق حساب الوسط الحسابي والخطأ المعياري. فحصت الدراسة الأرانب عند عمر ١,٥ شهر. أظهرت دراسة التغيرات الكمية في خلايا الذاكرة التائية وأنماطها الظاهرية زيادة في خلايا الذاكرة التائية المركزية والمستجيبة الرابعة والثامنة. أيضا، لوحظت اختلافات كمية في الأنماط الظاهرية لخلايا الذاكرة في الحيوانات المحصنة أوليا مقارنة بالتحصين المعزز. في أغلب الأحيان، يمكن رؤية الخلايا التائية الرابعة محددة والتي تنتج السيتوكينات في الخلايا اللمفاوية في الصفيحة العضلية اللفائفية والصائم. كان هناك اختلاف كبير في العدد العام للخصائص المنتجة للسيتوكينات بين المجموعة التي لم تخضع للتجربة والحيوانات المحصنة، حيث سيطرت عليها الخلايا التائية الرابعة في الأمعاء والغدد اللمفاوية. أظهرت هذه الدراسة العوامل الأساسية المساهمة في تطور ذاكرة الخلايا المناعية والمفيدة لمربي الحيوانات لغرض تطوير الذاكرة المناعية عند التلقيح.