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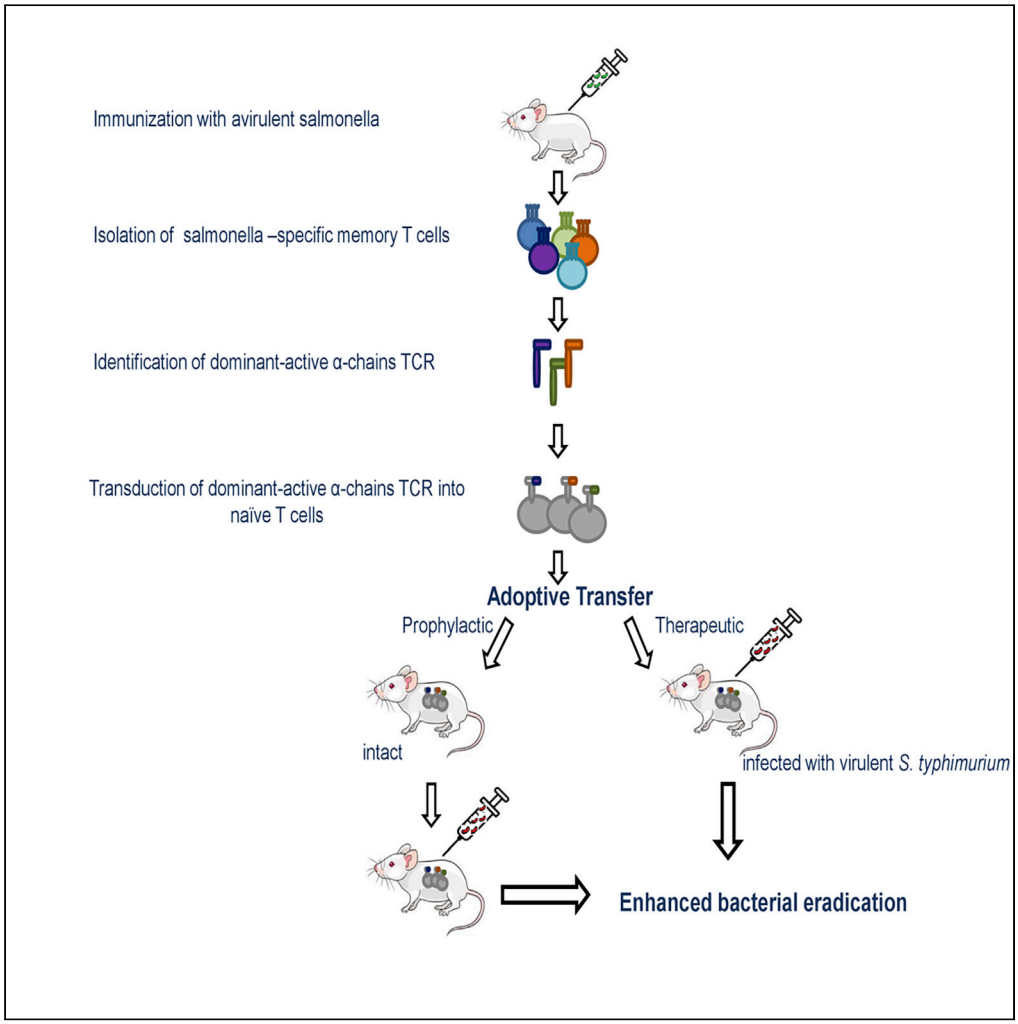

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HIGHLIGHTS

A regular TCR repertoire of memory T cells contains alpha-chain-centric TCRs

Dominant-active TCRα, paired with random TCRβ, recognizes specific microbial antigens

Adoptive immunotherapy could be applied for treatment of infections

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Adoptive Immunotherapy Based on Chain-Centric TCRs in Treatment of Infectious Diseases

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SUMMARY

Complications after vaccination, lack of vaccines against certain infections, and the emergence of antibiotic-resistant microorganisms point to the need for alternative ways of protection and treatment of infectious diseases. Here, we proposed a therapeutic approach to control salmonellosis based on adoptive cell therapy. We showed that the T cell receptor (TCR) repertoire of salmonella-specific memory cells contains 20% of TCR variants with the dominant-active α-chain. Transduction of intact T lymphocytes with the dominant salmonella-specific TCRα led to their enhanced *in vitro* proliferation in response to salmonella. Adoptive transfer of transduced T cells resulted in a significant decrease in bacterial loads in mice infected with salmonella before or after the adoptive transfer. We demonstrated that adoptive immunotherapy based on T cells, transduced with dominant-specific TCRα could be successfully applied for treatment and prevention of infectious diseases and represent a useful addition to vaccination and existing therapeutic strategies.

INTRODUCTION

Vaccination and antibiotics remain the most effective approaches for prophylaxis and control of infectious diseases. However, the emergence of antibiotic-resistant microorganisms, lack of vaccines against certain infections, safety concerns arising with the application of live attenuated strains or plasmid vaccines point out the need for the development of alternative prophylactic and therapeutic strategies. Here, we described an approach representing "vaccination without vaccines" based on adoptive immunotherapy using T cells, transduced with pathogen-specific T cell receptors (TCRs) that allows quickly forming a pool of effector T cells ready to fight infection. Moreover, here, we proposed an experimental technology for identification of therapeutic dominant-active α-chains, originated from chain-centric antigen-specific TCRs.

Adoptive immunotherapy has been discovered and successfully applied to treat patients with cancer (Zhang and Chen, 2018; Met et al., 2019). Gene modification of patient's lymphocytes with tumor-specific TCRs or chimeric antigen receptors (CARs) could significantly improve the efficiency of the adoptive cell therapy as it was demonstrated for treatment of melanoma, synovial sarcoma, colorectal cancer, etc. using TCR-modified lymphocytes or lymphomas using CAR-transduced T cells (Met et al., 2019). However, adoptive immunotherapy based on TCR-modified T cells remains expensive so far (Rosati et al., 2017) because of time- and labor-consuming processes of T cell cloning, sequencing of both TCR α- and β-chains, and controlling of their pairing. In this respect, focusing on chain-centric TCRs, i.e. identification of a dominant-specific chain (either TCRα or TCRβ) (Yokosuka et al., 2002; Nakatsugawa et al., 2015; Ochi et al., 2015; Zhao et al., 2016) could significantly lower costs and benefit the overall efficiency of adoptive cell therapy.

Previously, we demonstrated the dominant role of TCRα in recognition of the tumor alloantigen (Zamkova et al., 2019) that was consistent with other findings (Yokosuka et al., 2002; Nakatsugawa et al., 2015). Although several studies pointed out β-chain-centric TCRs (Ochi et al., 2015; Zhao et al., 2016), we assume that the leading role of TCRα in antigen recognition could be a regular phenomenon. It is known that during the thymic development, TCRα genes undergo editing to produce an α-chain that can not only successfully pair with a rearranged β-chain but also form a TCR with which a thymocyte will survive the positive selection (Huang et al., 2005; Hale and Fink, 2010). Furthermore, due to the lack of α-chain allelic exclusion, some mature T cells can express two TCR α-chains (Padovan et al., 1993) and, hence, two TCRs, allowing to

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avoid skewing the TCR repertoire specificity and immunodeficiency that arise in β-chain transgenic animals (Silaeva et al., 2014). Structural analyses of TCR - peptide/major histocompatibility complex (pMHC) complexes demonstrated that TCRα could dictate the orientation of the whole trimolecular complex and have more contacts with a pMHC (Stadinski et al., 2011), influencing TCRβ interaction with a pMHC and changing the TCR specificity (Yokosuka et al., 2002).

In studies here, we aimed to prove that dominant antigen-specific α-chain-centric TCRs frequently generate during the immune response. Moreover, we interested if adoptive immunotherapy could be applied for prevention and treatment of infectious diseases. For this, induction of salmonellosis in mice was used as an experimental infectious model.

Salmonella infections remain a serious health problem worldwide for both human and animals (Ahmad, 2002). Salmonella infections can cause in human local intestinal diseases (by nontyphoid serovars) or typhoid fever (by *S. enterica* serovars Typhi and Paratyphi) and pose the significant mortality threat (Wick, 2011; Griffin and McSorley, 2011; Santos, 2014; Behnsen et al., 2015). Although the innate and humoral immune responses are required to control salmonellosis (Cummings et al., 2009; Griffin and McSorley, 2011; Galen et al., 2016; Benoun et al., 2018), the T cell response is a crucial factor of immunity to salmonella, required for both eradication of the primary infection and establishment of protection against re-challenge (Mittrücker et al., 2002; Cummings et al., 2009; Benoun et al., 2018). Vaccination with live attenuated bacteria strains is considered as one of the promising approaches to provide for the host defense to the salmonella infection (Galen et al., 2016; Benoun et al., 2018). However, unlike other enteric pathogens, infection with salmonella does not induce a long-term protective immunity, and the candidate vaccines developed to date have moderate protective efficacy (Levine, 2006; Galen et al., 2016; Benoun et al., 2018). In this respect, a search of alternative preventive and therapeutic strategies to improve recovery from salmonellosis is of particular practical importance.

Here, we described an experimental approach to control salmonellosis based on adoptive transfer therapy. Using a murine model of *in vivo* establishment of memory T cells, specific to salmonella antigens (Lo et al., 1999), we generated salmonella-specific memory T cells and created cDNA libraries of their TCRs. In this work, we selected therapeutic TCRs based on the chain centricity of TCRs on the part of antigen-specific T lymphocytes (Nakatsugawa et al., 2015; Zamkova et al., 2019). This approach allowed us to identify TCR α-chains capable to form antigen-specific TCRs after pairing with various endogenous β-chains arisen in the T cell repertoire of a secondary host. By practicing this approach, we lost a part of the antigen-specific TCRs but greatly benefited in the time of identification of the desired TCR, avoiding T cell cloning or cloning of TCR genes from individual T cells. We showed that nearly 20% of the memory cell TCR repertoire, formed during the primary *in vivo* immune response to salmonella comprised of α-chain-centric TCRs. Our data demonstrated that *ex vivo* transduction of naive murine T lymphocytes with the single dominant-active salmonella-specific TCRα of memory T cells followed by the adoptive transfer to the host, infected with the virulent strain of *S. enterica* serovar Typhimurium facilitated reduction of bacterial loads. Moreover, the adoptive transfer of transduced T cells to naive mice provided for the protective effect against *S. typhimurium*.

Here, we demonstrated for the first time the possibility to operatively create efficient protective immunity without either vaccination or induction of the primary immune response in a recipient as adoptively transferred TCRα-transduced T cells could immediately fight the pathogen. This could represent a versatile therapeutic approach to control various infectious diseases. This strategy may be particularly important in challenging with antibiotic-resistant strains and fast-spreading infections or microorganisms for which there are no vaccines.

RESULTS

The general scheme of experiments is represented in Figure S1.

Generation of Memory T Cells Specific to S. typhimurium

Here, we aimed to generate salmonella-specific memory T cells to subsequently create cDNA libraries of their TCRs and search dominant-active salmonella-specific α-chains of these TCRs.

For this, B10.D2(R101) mice were i.p. immunized with *S. typhimurium* avirulent strain 247, and 3 weeks later, we analyzed functional activity of their splenocytes by *in vitro* stimulation with 2.0 x 10^3 - 2.0 × 10^7 CFU of heat-inactivated *S. typhimurium* virulent strain IE 147 for 72 h (Figure 1A). We observed 2.3-, 3.1-, and

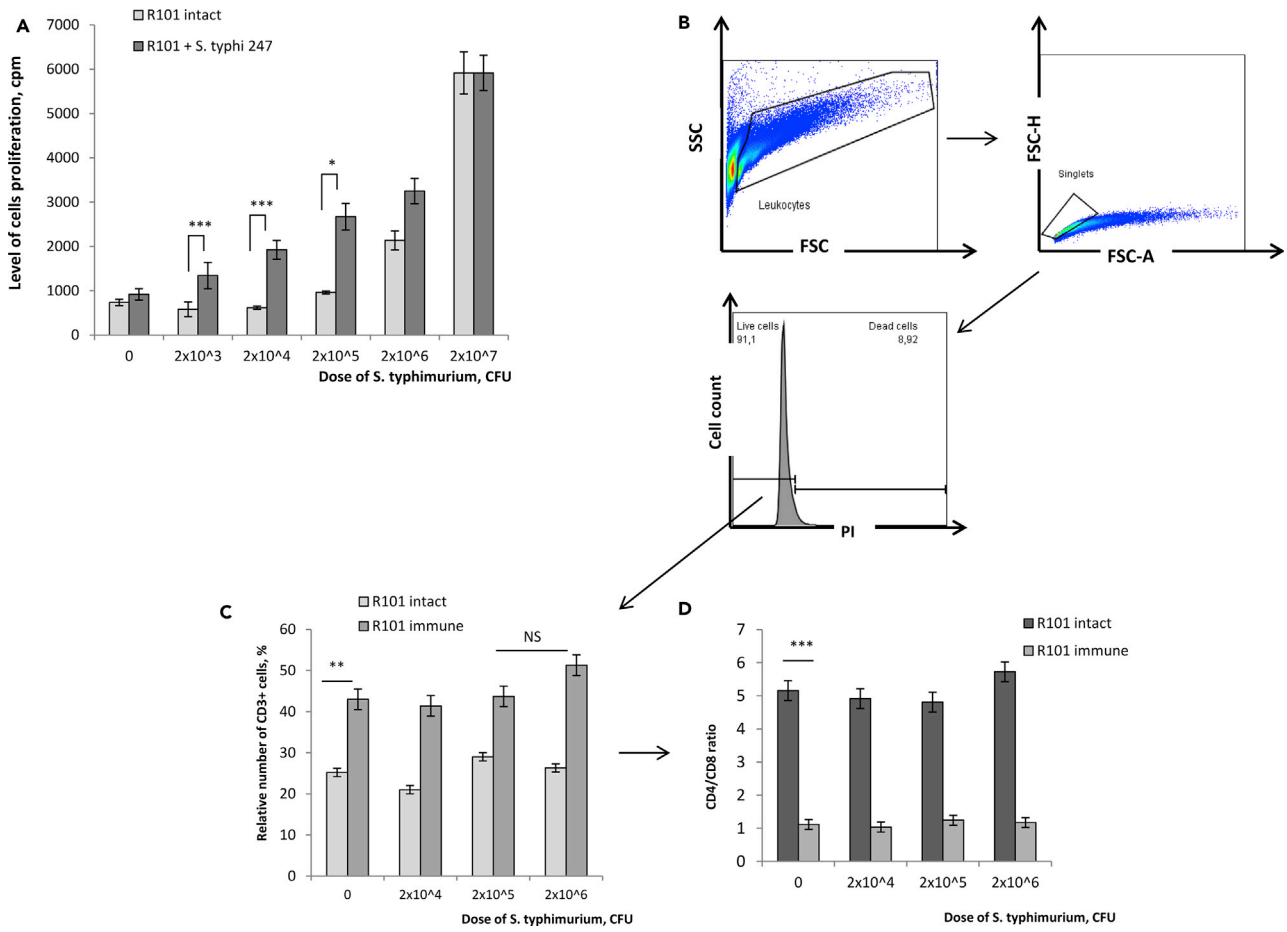

Figure 1. Functional and Phenotypic Characteristics of Salmonella-Specific Memory T Cells

B10.D2(R101) mice were i.p. immunized with *S. typhimurium* avirulent strain 247, and 3 weeks post-immunization total spleen cells of immunized mice were stimulated *in vitro* with increased doses of heat-inactivated *S. typhimurium* virulent strain IE 147 for 72 h. Total spleen cells of intact (non-immunized) B10.D2(R101) mice were used as the background control.

(A) Analysis of the salmonella-induced *in vitro* proliferative response.

(B–D) Flow cytometry analyses of lymphocytes 72 h post *in vitro* stimulation. (B) Cells were gated as follows: leukocytes were gated based on SSC-A vs. FSC-A, and then singlets were gated based on FSC-H vs. FSC-A, and dead cells were excluded by staining with PI. (C) The relative number (%) of T cells (CD3+) in the population of live singlet leukocytes. (D) CD4/CD8 T cells ratio in the population of CD3+ live singlet leukocytes.

Data are representative of 2 independent experiments and shown as mean ± SD (n = 3). *p < 0.03, **p < 0.01, ***p < 0.001 (unpaired Student's t-test). NS, not significant. See also Figures S1 and S4.

2.8-fold increased cell proliferation of immunized mice in response to 2.0 x 10^3, 2.0 x 10^4, and 2.0 × 10^5 CFU *S. typhimuriun*, respectively, as compared to non-immunized (intact) mice (Figure 1A). Further increase of the bacterial dose to 2.0 × 10^6 CFU resulted only in 1.5-fold enhanced proliferation of splenocytes of immunized mice as compared to the control. At the highest dose of *S. typhimuriun* (2.0 × 10^7 CFU), no differences in cell proliferation could be detected in both experimental groups (Figure 1A), suggesting possible involvement of T cell independent salmonella antigens in the proliferative response. Thus, this dose of salmonella was not subsequently used in *in vitro* studies here.

Next, we analyzed the relative count of $CD3^+$ lymphocytes and $CD4^+$ and $CD8^+$ T cell subsets in the cultures of splenocytes of immunized mice 72 h post-restimulation *in vitro* with bacterial cells (Figures 1B–1D). Spleen cells of intact mice, similarly cultured were used as the background control. Flow cytometry analyses showed similar cell viability in all culture conditions that averaged to 90% (Figure 1B). Immunization of B10.D2(R101) mice with avirulent strain 247 resulted in the 2.0-fold increased relative counts of T lymphocytes in their spleen as compared to intact control mice (Figure 1C(0)) and the 5.0-fold decreased CD4/CD8 ratio (Figure 1D(0)), indicating dramatic accumulation of CD8 T cells. However, *in vitro* restimulation of

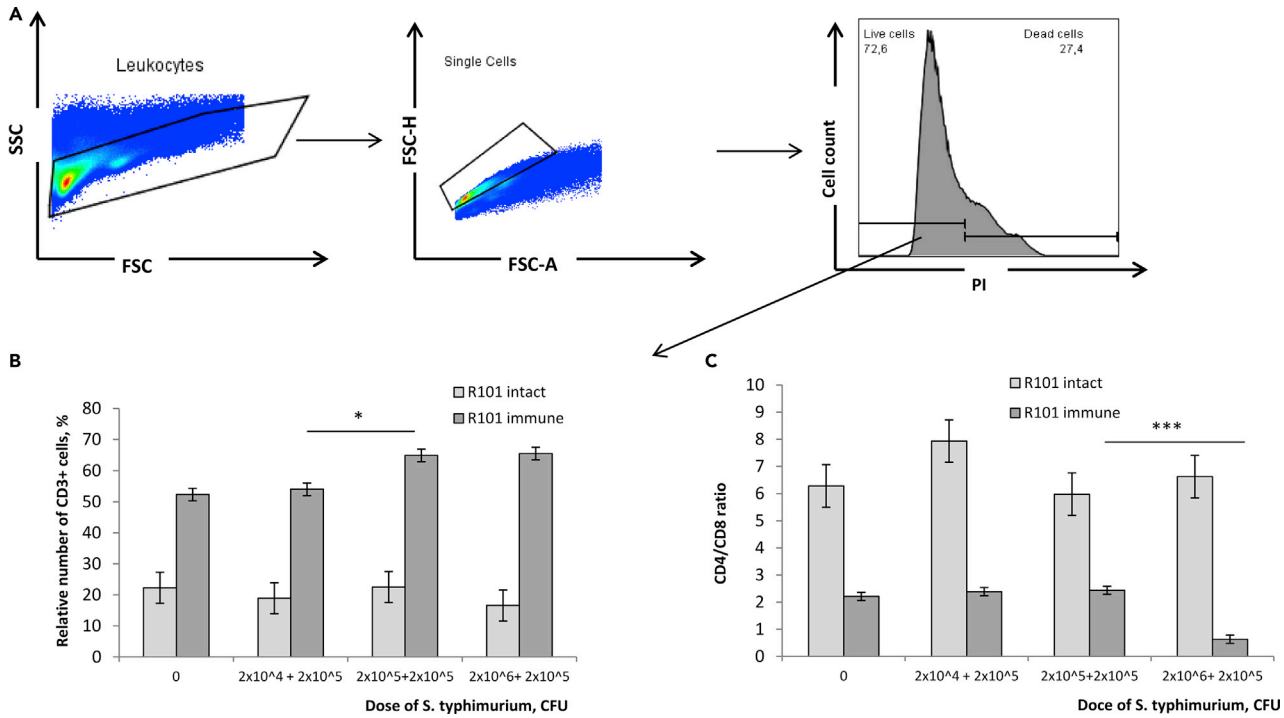

Figure 2. Phenotypic Characteristics of T Cells Post Two *in vitro* re-Stimulations by Salmonella

B10.D2(R101) mice were immunized with *S. typhimurium* avirulent strain 247, and 3 weeks later their total spleen cells were stimulated *in vitro* with heat-inactivated *S. typhimurium* virulent strain IE 147 for 72 h. Cells were then additionally re-stimulated for another 72 h followed by the flow cytometry analyses. Total spleen cells of intact B10.D2(R101) mice, similarly cultured for 6 days were used as a control.

(A) Leukocytes were gated based on SSC-A vs. FSC-A, and then singlets were gated based on FSC-H vs. FSC-A, and dead cells were excluded by staining with PI.

(B) The relative number (%) of T cells (CD3+) in the population of live singlet leukocytes.

(C) CD4/CD8 T cells ratio in the population of CD3+ live singlet leukocytes.

Data are representative of 2 independent experiments and shown as mean ± SD (n = 3). *p < 0.05, ***p < 0.001 (unpaired Student's t-test).

splenocytes of immunized mice with *S. typhimurium* did not lead to any further changes in the relative number of $CD3^+$ cells (Figure 1C) and CD4/CD8 ratios (Figure 1D).

To enrich the pool of salmonella-specific memory cells, next, we performed two rounds of *in vitro* restimulation. For this, spleen cells of immunized animals were cultured with 2.0 x 10^4 - 2.0 × 10^6 CFU of heat-inactivated virulent strain IE 147 for 72 h followed by the second restimulation with 2.0 × 10^5 CFU for another 72 h (Figure 2). Cell viability on Day 6 of culture approximated to 70% (Figure 2A). We observed 1.2- fold increase in the relative number of CD3+ lymphocytes in cell cultures with 2.0 x 10^5 - 2.0 x 10^6 + 2.0 × 10^5 CFU of salmonella (Figure 2B). Moreover, double stimulation of splenocytes of immunized mice with 2.0 x 10^6 + 2.0 × 10^5 CFU *S. typhimurium* led to the proliferation of CD8 T cells that resulted in 4.0-fold decrease of the CD4/CD8 ratio as compared to lower bacterial doses (Figure 2C). Hence, for the subsequent generation of cDNA libraries of memory cells TCRs, we enriched salmonella-specific memory T cells *in vitro* as follows: co-culture with 2.0 × 10^6 CFU of heat-inactivated *S. typhimurium* virulent strain IE 147 for 72 h followed by the second restimulation with 2.0 × 10^5 CFU for another 72 h.

Screening of α-Chains TCR In Vitro

Based on the analyses of the generated cDNA libraries (see Transparent Methods), we selected 23 enriched T cell clones with 1.5–3.5-fold increased frequency after the second *in vitro* restimulation by salmonella as compared to the pool of memory T cells without *in vitro* restimulation (Table 1).

T cells expressing the transgenic variants of TCRα of these 23 memory T cell clones were generated by the retroviral transduction and cultured *in vitro* with antigen-presenting cells (APCs), loaded with heat-inactivated *S. typhimurium* virulent strain IE 147 (see Transparent Methods). The level of cell proliferation in cultures with

TCRα	CDR3 Amino Acid Sequence	V Segment	J Segment	T Clone Frequency, %	
				Without *In Vitro* Re-stimulation	Post *In Vitro* Re-Stimulation
SM1	CAARVAGYNKLTF	TRAV14D-1	TRAJ11	0.003	0.008
SM3	CAASAVDTGYQNFYF	TRAV14D-3-DV8	TRAJ49	0.002	0.007
SM4	CVVGKTGFASALTF	TRAV11	TRAJ35	0.002	0.007
SM5	CALSDDSGYNKLTF	TRAV12-1	TRAJ11	0.002	0.006
SM6	CAASSGTYQRF	TRAV14D-3-DV8	TRAJ13	0.003	0.006
SM7	CATVMSNYNVLYF	TRAV8-1	TRAJ21	0.002	0.004
SM9	CAVNNAGAKLTF	TRAV3-1	TRAJ39	0.002	0.004
SM10	CVRSLSDTNAYKVIF	TRAV6-2	TRAJ30	0.002	0.003
SM11	CALSDLPNTNKVVF	TRAV6-7-DV9	TRAJ34	0.002	0.003
SM12	CAVSAWTNTNKVVF	TRAV9-1	TRAJ34	0.002	0.003
SM13	CAVSVMDSNYQLIW	TRAV3-1	TRAJ33	0.002	0.003
SM14	CAVSAWGNTGKLIF	TRAV9-1	TRAJ37	0.001	0.002
SM15	CAVSPVNTGNYKYVF	TRAV7-5	TRAJ40	0.001	0.002
SM16	CAMREINQGGSAKLIF	TRAV16N	TRAJ57	0.001	0.002
SM17	CAAEGDDNNNAPRF	TRAV4-3	TRAJ43	0.001	0.002
SM18	CALSGSGGSNAKLTF	TRAV12-3	TRAJ42	0.001	0.002
SM19	CAMREGTSSFSKLVF	TRAV16D-DV11	TRAJ50	0.001	0.002
SM20	CAVITASLGKLQF	TRAV3-1	TRAJ24	0.005	0.01
SM21	CAMREVMDSNYQLIW	TRAV16N	TRAJ33	0.003	0.007
SM22	CAVSAWDNNNAPRF	TRAV9-1	TRAJ43	0.001	0.002
SM23	CAASAGSNYNVLYF	TRAV5-4	TRAJ21	0.001	0.002
SM24	CAVSMRSGSFNKLTF	TRAV9N-3	TRAJ4	0.002	0.003
SM27	CAASVNYGNEKITF	TRAV5-4	TRAJ48	0.001	0.002

Table 1. Clone Frequencies in cDNA Libraries of Salmonella-Specific Memory T Cells
See also Tables S1 and S2.

unloaded APC was used as the background control. The level of the antigen-induced proliferative response of T cells, transduced with TCRα was measured in 72 h and compared to the proliferation levels of non-transduced T cells (NTCs) and GFP-transduced lymphocytes (negative controls) (Figure 3). We considered TCRα as a dominant-specific to the *S. typhimurium* antigens if the level of the antigen-induced proliferation of respective transduced T cells was at least 2.0-fold higher than of the negative controls. Screening *in vitro* revealed 5 such functionally dominant-active variants of TCRα: SM1, SM14, SM16, SM20, and SM21 (Figure 3).

Physicochemical Properties of the CDR3 Region of Dominant-Active Salmonella-Specific TCRα

Analyses of the amino acid (AA) sequences in the CDR3 region of 5 functional dominant-active salmonella-specific TCRα (SM1, SM14, SM16, SM20, and SM21) and evaluation of their physicochemical properties were performed using VDJ tools (Shugay et al., 2015).

CDR3 of TCRα SM16 and SM21 contained 5 hydrophilic AAs (41.7% and 45.4%, respectively), whereas CDR3 of SM1, SM14, and SM20 TCRα had only 2 and 3 hydrophilic AAs, respectively (33.3%, 20.0%, and 22.2%,

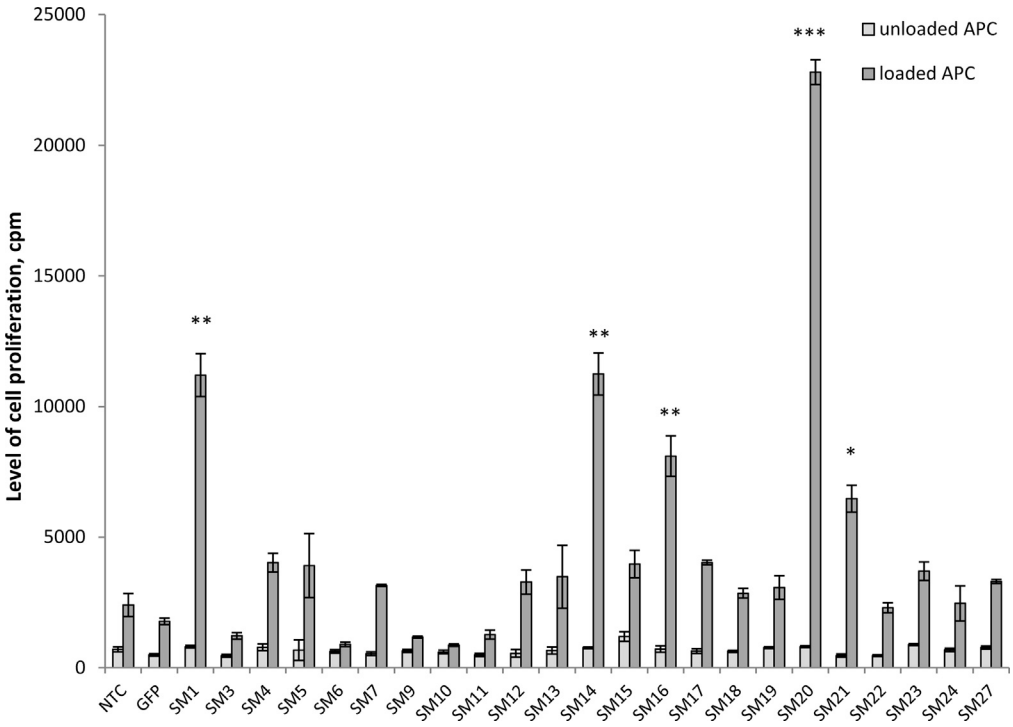

Figure 3. *In vitro* Screening of α-Chains TCR

Lymphocytes of intact B10.D2(R101) mice were transduced with the indicated TCRα or GFP and cultured *in vitro* for 72 h with unloaded antigen-presenting cells (APCs) (to assess the background proliferation) or with APC, loaded with heat-inactivated *S. typhimurium* virulent strain IE 147 (to assess the antigen-induced proliferation). The level of antigen-induced proliferation of non-transduced lymphocytes (NTCs) was used as the reference. Data are representative of one of 2 independent experiments and shown as mean ± SD for three technical repeats. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ (unpaired Student's t-test). See also Figures S1, S2, and S5, Tables S1, and S3.

respectively) (Figure S2A). Furthermore, only SM16 and SM21 contained negatively charged AA (1 and 2, respectively) in their CDR3α region (Figure S2B). Importantly, SM16 and SM21 TCRα belong to the same Va16 family, as determined by the analyses of generated cDNA libraries (Table 1).

The middle part of CDR3 (specifically, 5 central AAs) often contacts with the presented antigen (Egorov et al., 2018), and its physicochemical characteristics could influence the properties of the whole CDR3 region. Thus, next, we analyzed the averaged values of strength, hydropathicity, and polarity for the central 5 AAs of the CDR3α (cCDR3α) of each TCRα (Table S1). The cCDR3α of all dominant-active TCRα, except for SM16, contained one strongly interacting AA (Table S1) that could form hydrophobic interactions, presumably enhancing TCR binding affinity (Egorov et al., 2018; Lu et al., 2019). According to GRAVY, only the cCDR3α of SM1 and SM20 TCRα were hydrophobic (Table S1), with cCDR3α of SM20 having the highest hydrophobic index. Interestingly, naive T cells, transduced with this SM20 TCRα exhibited the highest proliferative potential *in vitro* in response to salmonella antigens (Figure 3). However, we could not find any correlation between the cCDR3α GRAVY and the proliferative potential of the corresponding TCRα-transduced T cells for other four salmonella-specific dominant-active TCRα (Table S1, Figure 3). The mean polarity of cCDR3α of all five dominant-active TCRα was comparable (Table S1).

Phenotypic Characteristics of T Lymphocytes, Transduced with Salmonella-Specific TCRα

To prove the functional activity of the selected 5 variants of TCRα, we performed several *in vivo* adoptive transfer experiments. But first, we analyzed the phenotypic characteristic of transduced T cells on Day 3 post-transduction.

Activation and expansion of spleen cells *in vitro* in the presence of ConA and IL-2 resulted in accumulation of >90% CD3+ T lymphocytes (Figure 4A). Transduction of T cells with either salmonella-specific TCRα SM1

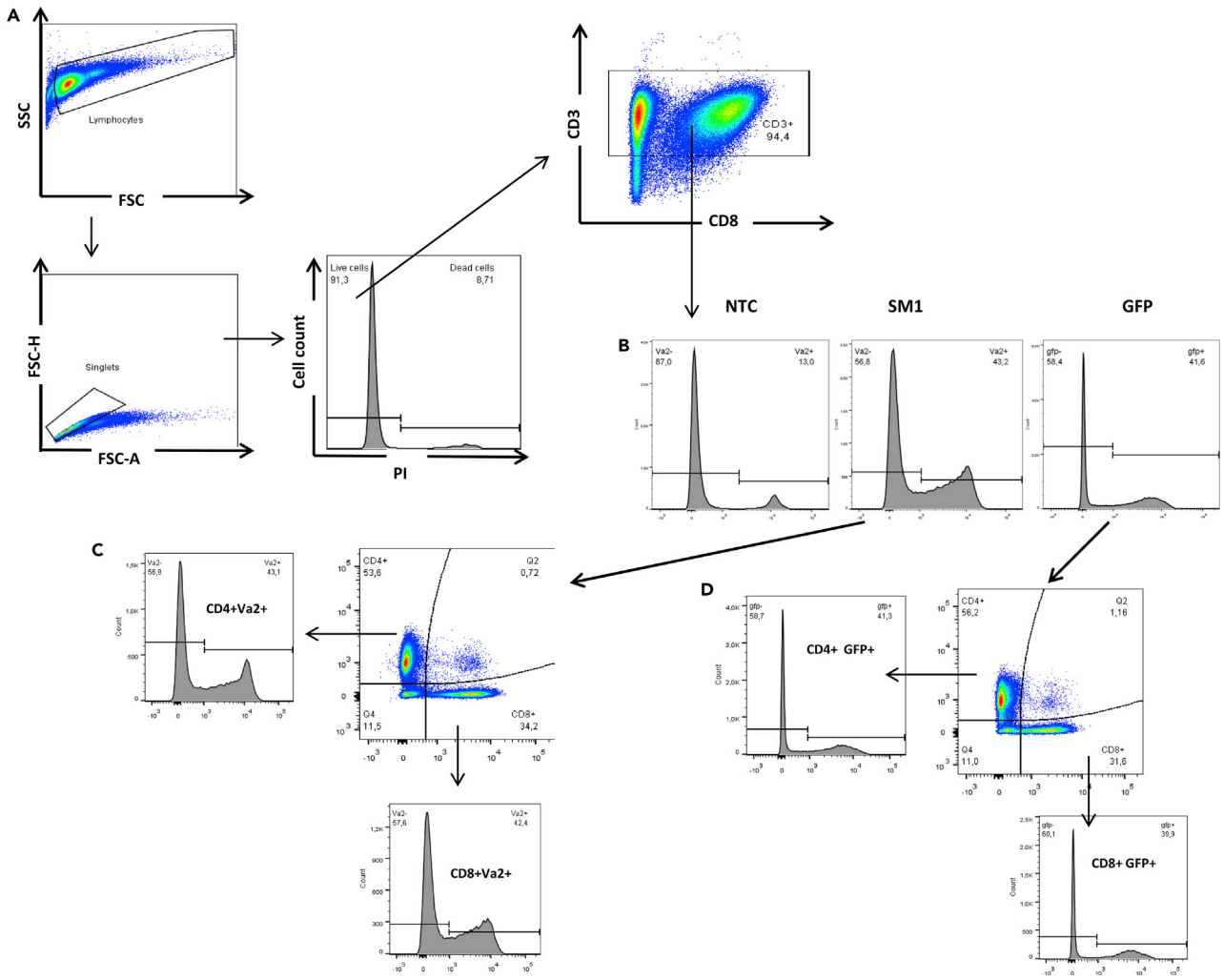

Figure 4. Flow Cytometry Analyses of CD3+ T Cells Prior to Adoptive Transfer

Splenocytes of intact B10.D2(R101) mice were activated with ConA (3 μg/mL) and IL-2 (10 U/mL) for 24 h and transduced with SM1 TCRα or GFP. Flow cytometry analyses were performed on Day 3 post-transduction (6 days *in vitro* cultivation in total). Non-transduced cells (NTC), similarly cultured for 6 days were used as the control.

(A) Cells were gated as follows: lymphocytes were gated based on SSC-A vs. FSC-A followed by singlets gating based on FSC-H vs. FSC-A. Dead cells were excluded by staining with PI. The relative count of CD3+ cells were determined within the population of live singlets after staining with anti-CD3 monoclonal antibodies.

(B) The level of transduction measured by anti-Va2 staining for TCRα SM1 or by GFP expression (used as the reference) in the population of CD3+ live singlets.

(C) The relative number of CD4+ and CD8+ cells in the subset of SM1 transduced T cells. The relative count of $V\alpha2^+$ cells were analyzed in CD4+ and CD8+ T cells subsets.

(D) The relative number of CD4+ and CD8+ cells in the subset of GFP transduced T cells. The relative count of GFP^+ cells were analyzed in CD4+ and CD8+ T cells subsets. One of two representative experiments is shown (n = 2). Data are presented as mean ± SD.

or GFP did not influence cell viability (over 90% on Day 6 of culture in total according to PI staining; Figure 4A).

Based on the AA sequences of the selected TCRα (Table 1), their corresponding Vα families were determined (Table S2). To assess the efficiency of transduction with SM1 TCRα, the relative number of $V\alpha2^+$ cells was measured in the SM1-transduced T cells culture and compared with the counts of $V\alpha2^+$ cells in the NTC culture (Figure 4B). We observed at least a 3.0-fold increase of $V\alpha2^+$ cells in the population of SM1-transduced T lymphocytes as compared to the NTC control. Of particular note, the efficiency of SM1 TCRα transduction corresponded well with the level of transduction with GFP (Figure 4B).

As there are no commercially available antibodies to Vα3, Vα5, and Vα16 families, the efficiency of transduction with the respective TCRα was determined using a level of the reporter expression in the culture of GFP-transduced cells as the reference. Levels of transduction were 40 - 70%.

Analyses of transduced T cells showed that modification with either SM1 TCRα or GFP resulted in the comparable CD4/CD8 ratio, and SM1 TCRα or GFP expressed similarly in both $CD4^+$ and $CD8^+$ T lymphocytes (Figures 4C and 4D). Thus, in adoptive transfer experiments here, we used modified T lymphocytes composed of $CD4^+$ and $CD8^+$ cells at the ratio 3:2, both expressing the transduced TCRα.

Evaluation of the Therapeutic Potential of Salmonella-Specific TCRα In Vivo

The salmonella infection is naturally spread by the oral route. Thus, in all studies here, we infected experimental animals with 4.0–6.0 x 10^6 CFU/mouse (LD27) (see Transparent Methods) of *S. typhimurium* virulent strain IE 147 per os via a gavage needle. To assess the therapeutic potential of salmonella-specific TCRα *in vivo*, T lymphocytes, transduced with either TCRα SM1, SM16, or SM21 were independently i.v. injected in a dose of 3.0 x10^5 cells/mouse to mice on Day 3 post-infection. Control NTC was similarly transferred to an independent experimental group as the negative control. Bacterial loads in the spleen of mice were analyzed on Day 7 post-infection, i.e. before any animal mortality could occur (Figure S3B).

The adoptive transfer of NTC to infected animals resulted in a 1.4-log decrease in the bacterial loads as compared to mice without the adoptive transfer (Figure 5). These data clearly indicated that polyclonal activated (by ConA and IL-2) T cells are involved in the immune response to salmonella and could provide the significant therapeutic effect by themselves. Thus, in all experiments here, the NTC group was used as the main control for groups with the adoptive transfer of T cells, transduced with salmonella-specific TCRα.

The adoptive transfer of T cell transduced with SM1, SM16, or SM21 did not lead to a further decrease of bacterial loads in the spleen of mice as compared to the NTC group (Figures 5A and 5B). Next, we decided to inject a mixture of transduced T cells. Lymphocytes, independently transduced with TCRα SM1, SM14, SM16, SM20, or SM21 were randomly divided into 2 groups and equally mixed as follows: Mix 1 (SM16 + SM21) and Mix 2 (SM1 + SM14 + SM20). These two mixtures of transduced T cells were i.v. transferred to infected animals (the total dose of T cells was 3.0 x 10^5 cells/mouse). We observed the significant 1.8 -log decrease in the bacterial loads in the spleen of mice with adoptively transferred Mix 1 (SM16 + SM20) as compared to the NTC control and 3.24-log decrease as compared to animals without the adoptive transfer (Figures 5C and 5D). Moreover, the adoptive transfer of transduced T cells SM16 + SM21 resulted in complete salmonella eradication in 36% of mice (9 of 25) by Day 7 post-infection (Figure 5C). However, no therapeutic effect was observed with the adoptive transfer of Mix 2 (SM1 + SM14 + SM20) as compared to the NTC group (Figures 5C and 5D).

Our data demonstrated that co-transfer of several types of T lymphocytes, independently transduced with the single salmonella-specific TCRα could improve control of the salmonella infection and resulted in enhanced elimination of the bacteria.

Analysis of the Prophylactic Potential of Salmonella-Specific TCRα In Vivo

Previously, we demonstrated that transduced T cells, adoptively transferred to a naive host could persist in the recipient's organism and exhibit their functional activity within 14 days post-transfer (Zamkova et al., 2019). In this respect, we assumed that the adoptive transfer of T cells, transduced with salmonella-specific TCRα could provide protection from salmonellosis to a naive host.

In order to assess the prophylactic potential of salmonella-specific TCRα *in vivo*, T cells independently transduced with TCRα SM1, SM14, SM16, SM20, or SM21 were divided into two groups, equally mixed and i.v. injected to intact B10.D2(R101) mice as described above. These mice were infected with 4.0–6.0 x 10^6 CFU/mouse (LD27) of *S. typhimurium* virulent strain IE 147 on Day 3 post - adoptive transfer. The analysis of the bacterial load in the spleen of mice was performed on Day 7 post-infection. Again, we observed the significant 0.95-log decrease in the bacterial loads in the spleen of mice, received NTC as compared to mice without the adoptive transfer (Figure 6). Co-transfer of transduced T cells SM1+ SM14 + SM20 (Mix 2) resulted in the significant 1.83-log decrease in the CFU number as compared to the NTC control and 2.72-log decrease as compared to animals without the adoptive transfer (Figure 6). Moreover, in 30% of mice (5

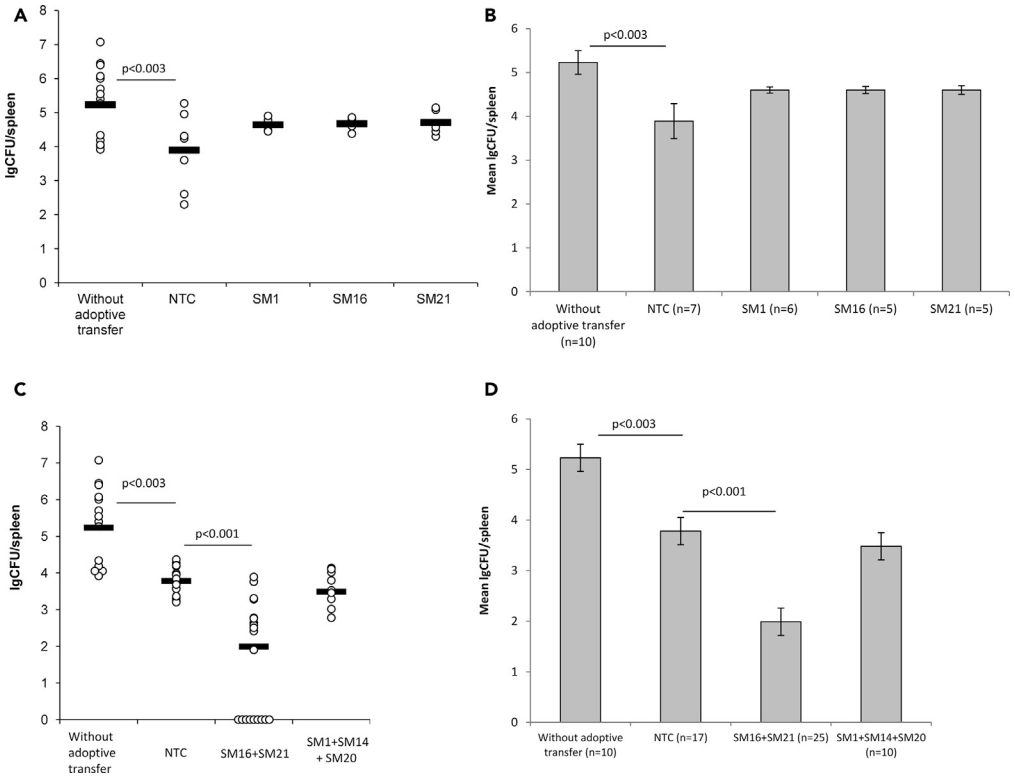

Figure 5. Therapeutic Potential of Transduced T Cells, Adoptively Transferred to Infected B10.D2(R101) Mice

B10.D2(R101) mice were enterogastrally infected with LD27 (4.0–6.0 x 10^6 CFU/mouse) of the *S. typhimurium* strain IE 147. On Day 3 post-infection, mice were i.v. injected with either non-transduced cells (NTC) or T cells, transduced with the single salmonella-specific TCRα in the dose of 3.0 x 10^5 cells/mouse. Infected mice without the adoptive transfer were used as the control. The log number of bacterial colony-forming units (lgCFUs) was counted in the spleen on Day 7 post-infection.

(A and B) Adoptive transfer of individual types of T cells, transduced with either single TCRα SM1, SM16, or SM21.

(C and D) Adoptive co-transfer of individual types of T cells, transduced with the single TCRα as specified. (A and C) The lgCFU number per spleen. (B and D) The mean lgCFU number per spleen.

Data are presented as mean ± SD. One-way ANOVA, corrected with Tukey's multiple comparisons. See also Figures S1, S3, and S4.

of 15) complete bacteria eradication was achieved (Figure 6A). No prophylactic potential of T cells, modified by α-chains TCR SM16 or SM21 (Mix 1), was demonstrated in this experimental system (Figure 6).

It seems possible that further optimizations of the method by parameters of doses of adoptively transferred cells and/or time frame between the adoptive transfer and infection would increase the protective potential of T cells transduced with salmonella-specific TCRα.

DISCUSSION

In studies here, we proposed an experimental technology for identification and application of individual α-chains TCR, originated from alpha-chain-centric TCRs (Yokosuka et al., 2002; Nakatsugawa et al., 2015; Ochi et al., 2015; Zhao et al., 2016). Such TCRα, paired with various β-chains can dictate the same TCR specificity (Yokosuka et al., 2002; Nakatsugawa et al., 2015; Zamkova et al., 2019). Previously, we showed that TCRα of memory T cells determines the TCR specificity to the tumor antigen both in the TCRα transgenic mice model and when transduced into naive T lymphocytes of wild-type mice (Zamkova et al., 2019). Here, we confirmed that a regular TCR repertoire contains α-chain-centric TCRs and demonstrated that such dominant-active TCRα could be used for adoptive cell therapy and protection from infectious disease.

Adoptive immunotherapy is mainly applied for the treatment of cancer. The possibility of its use for prevention and treatment of infectious diseases has been declared, but no direct data have been obtained. Here,

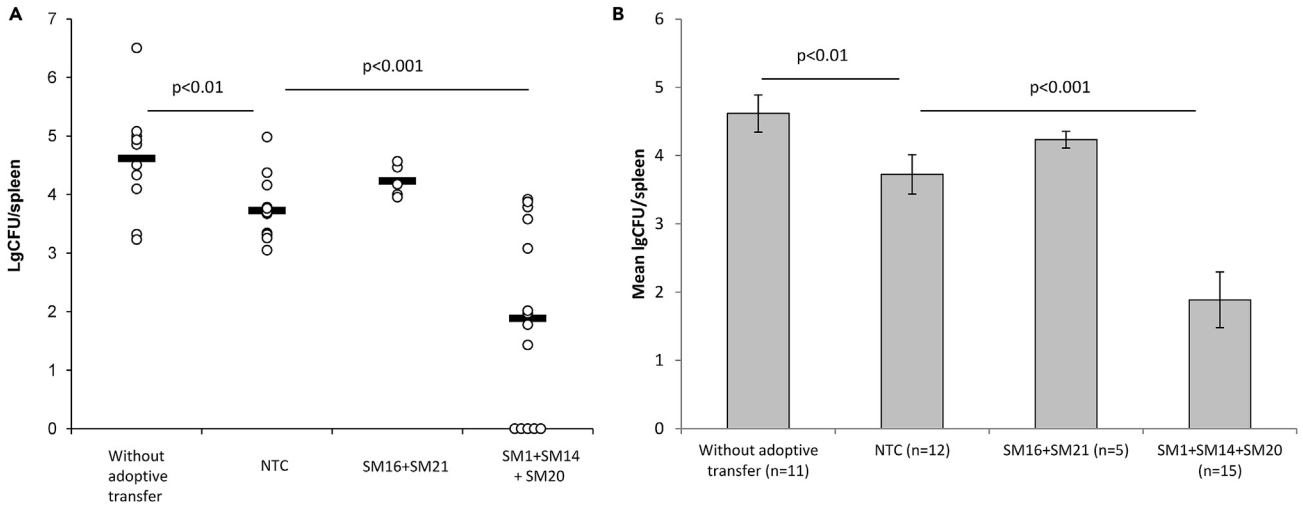

Figure 6. Prophylactic Potential of Adoptive Transfer of T Cells, Transduced with the Single Salmonella-Specific TCRα

Intact B10.D2(R101) were i.v. injected with either non-transduced T cells (NTCs) or mixture of individual types of T cells, transduced with the single TCRα as specified. On Day 3. post-adoptive, transfer mice were enterogastrally infected with LD27 (4.0–6.0 x 10^6 CFU/mouse) of *S. typhimurium* strain IE 147. Mice without the adoptive transfer were used as the control. The log number of bacterial colony-forming units (lgCFUs) was counted in the spleen of mice on Day 7 post-infection. (A) The lgCFU number per spleen.

(B) The mean lgCFU number per spleen. Data are presented as mean ± SD. One-way ANOVA, corrected with Tukey's multiple comparisons. NS, not significant. See also Figures S1, S3, and S4.

we employed *Salmonella typhimurium* as a model infection in mice and described an experimental therapeutic strategy to control salmonellosis based on the adoptive transfer of T lymphocytes, transduced with a single dominant-active TCRα of salmonella-specific memory T cells.

The enriched pool of salmonella-specific T lymphocytes was generated by *in vitro* re-stimulation of memory cells, established during the primary *in vivo* immune response to the avirulent *Salmonella* strain 247 (Lo et al., 1999). Analyses of TCRα cDNA libraries revealed 23 memory T cell clones with at least 1.5-fold increased frequency after *in vitro* re-challenge with salmonella. The single TCRα of all these clones were individually transduced into activated T lymphocytes of intact mice and challenged *in vitro* with salmonella antigens. T cells modified with 5 variants of TCRα exhibited a profound salmonella-specific *in vitro* response (Figure 3). Thus, we proved that TCRα can dominate in the antigen recognition by memory T cells and such α-chain-centric TCRs comprise the significant fraction (~20%) of the memory cells TCR repertoire.

Once established in mice by immunization with an attenuated vaccine strain (Lo et al., 1999; Mittrücker et al., 2002; Kirby et al., 2004), the salmonella-specific immune defense cannot be fully transferred to a naive host (Nauciel, 1990; Mastroeni et al., 1993; Benoun et al., 2018). Several reports demonstrated that the adoptive transfer of salmonella-specific T cell lines confers only partial defense to naive susceptible mice (Mastroeni et al., 1993; Paul et al., 1988). Adoptive transfer experiments here showed that T cells, transduced with a single salmonella-specific TCRα facilitated elimination of salmonella in mice with the established infection and contributed to complete bacteria eradication in over 30% of infected mice (Figure 5). Thus, the therapeutic adoptive transfer of transduced T cells could form the first line of protection in the acute phase of salmonellosis by creating a pool of the pathogen-specific effector T cells, ready to immediately fight the infection. These transferred salmonella-specific T lymphocytes could act before any adaptive immune response arises in the host, alleviating the infectious process and promoting the recipient's immune response.

Moreover, our data indicated that T cells, transduced with the dominant active salmonella-specific TCRα could render the host defense against salmonellosis. The adoptive co-transfer of several types of TCRα--modified T lymphocytes to a naive host improved control of salmonellosis and resulted in the rapid (by Day 7 post-infection) eradication of the bacteria in 30% of infected animals (Figure 6). We assume that such preventive adoptive transfer, aimed at multiple molecular targets of a pathogen, could be an effective addition to existing prophylactic measures for various infectious diseases.

Based on our recent data, obtained in the anti-tumor model system (Zamkova et al., 2019), we expect that transduced salmonella-specific T cells, adoptively transferred to the host either before or during the infection would not impair the formation of the host immunological memory to salmonella. Both adoptively transferred transduced T cells and the recipient's T lymphocytes could contribute to the formation of memory cells, thus, creating the enhanced immune protection against salmonella re-challenge.

Of particular note, both therapeutic and prophylactic effect in studies here was only achieved when several independently modified pools of T lymphocytes, transduced with the single salmonella-specific TCRα were co-transferred to mice (Figures 5 and 6). Although a relatively low number of immunizing antigens of *Salmonella* spp. have been determined and characterized to date (McSorley et al., 2000; Cummings et al., 2009), it seems likely that TCRs of salmonella-specific memory T cells possess specificity to different bacterial antigens. Interestingly, dominant-active TCRα SM16 and SM21 belong to one Vα16 family (Table 1) and share some common physicochemical features (Table S1, Figure S2). Hence, we proposed that TCRα SM16 and SM21 could recognize very similar salmonella antigens, whereas TCRα SM1, SM14, and SM20 that belong to various Vα families could presumably recognize a broader range of the antigens. Thus, cotransfer of several TCRα could expand the diversity of target salmonella antigens, recognized by transduced lymphocytes, hence, reducing the possibility of the pathogen escaping the immune response and improving the overall therapeutic efficiency.

Strikingly, in studies here, Mix 1 of transduced T cells (TCRα SM16 and SM21) with presumably more narrow antigen specificity provided therapeutic but no prophylactic effect upon adoptive transfer, whereas Mix 2 (TCRα SM1, SM14, and SM20) rendered the opposite effect (Figures 5 and 6). Taking this into account, we assumed that the availability of target antigens *in vivo* could also contribute to the efficacy of the adoptive transfer therapy here.

The therapeutic adoptive transfer was performed on Day 3 post-infection, i.e., in the acute phase of salmonellosis, when only surface-associated antigens of live salmonella could be available for the host immune system (Barat et al., 2012). These surface-associated antigens are abundant and tend to be low immunogenic (Barat et al., 2012). It seemed possible that TCRα SM16 and SM21 could recognize some of these abundant antigens, and the therapeutic adoptive transfer of Mix 1 could generate a large pool of specific effectors, ready to eliminate the infection. On the contrary, transduced T cells in Mix 2, although having a presumably wider range of the antigen specificity, could not find enough targets likely less presented by host cells to implement any significant therapeutic effect, when adoptively transferred in the acute phase of salmonellosis.

On the other hand, the preliminary adoptive transfer of Mix 2 could significantly broaden the repertoire of peripheral T cells, introducing a large pool of effectors with various specificity to salmonella antigens. It is known that shortly after salmonella infection, some bacterial mortality is observed (Grant et al., 2008), and internal antigens of killed salmonella could be released and presented to host T cells (Barat et al., 2012). Thus, the broader range of antigens, presented by live and killed bacterial cells in the initial phase of infection, could be recognized by ready effectors with the wider TCR repertoire, formed by the prophylactic adoptive transfer of Mix 2. Conversely, lack of sufficient specific targets for Mix 1 could make it ineffective in this experimental system.

It should be noted that in our *in vivo* experiments both therapeutic and prophylactic effects were observed when activated non-transduced T cells were transferred to infected or naive mice, respectively. *In vitro* culture of lymphocytes with ConA and IL-2, provided for in our adoptive transfer system, resulted in polyclonal activation of T cells and production of different cytokines (i.e., IL-2, IFNγ, IL-4) (Colle et al., 1993; Ruzek et al., 1996; Azadmehr et al., 2016). Thus, *in vivo* adoptive transfer of activated T cells could act as an adjuvant of the immune response of host T cells (Paul et al., 1985). It was also shown that cytokines alone could provide some degree of protection against the *S. typhimurim* infection (Fukazawa et al., 1983; Cummings et al., 2009). Earlier studies demonstrated that the inflammatory microenvironment within salmonella-infected tissues could provide activation stimuli to T cells in addition to a direct cognate TCR stimulation (Fukazawa et al., 1983). It is plausible that in studies here transferred polyclonal activated T cells with a lower activation threshold could be stimulated in a noncognate manner in infected tissues of mice and be involved in the host immune response to *S. typhimurium* (Pham and McSorley, 2015). However, it should be noted that despite of significant non-specific effects of non-transduced lymphocytes, observed in studies here the

adoptive transfer of dominant-specific TCRα-modified T cells could further improve the control of salmonellosis and benefited the overall therapeutic efficiency. Moreover, only immunotherapy with these transduced T cells resulted in complete bacteria eradication in 30% of mice, infected either before or post-adoptive transfer. This effect was not observed when using non-transduced control lymphocytes.

It is well recognized that both CD4 and CD8 T cells are required for the effective adaptive immune response to *S. typhimurim* (Paul et al., 1988; Mittrücker et al., 2002; Cummings et al., 2009; Behnsen et al., 2015). In studies here, we performed the adoptive transfer of transduced T cells, comprised both CD4 and CD8 cells that equally expressed the salmonella-specific TCRα (Figures 4C and 4D). As salmonella replicates in phagocytic vacuoles of infected host cells, CD4 T cells are essential to control salmonellosis via production of the superoxide radical in infected cells (Hess et al., 1996; McSorley et al., 2000). *S. typhimurium* induces a strong T-helper 1 (Th1) response in mice, and Th1 cytokines (IFNγ, TNFα) activate bactericidal mechanisms in macrophages (McSorley et al., 2000; Mittrücker and Kaufmann, 2000). CD4 T cells are required for salmonella-specific antibody production, providing help for B cell isotype switching and affinity maturation (Mittrücker et al., 2002; Cummings et al., 2009). CD4 T cells also take part in granuloma formation that controls the bacteria dissemination (Mittrücker and Kaufmann, 2000). Establishment and maintenance of salmonella-specific CD8 response is also Th1-dependent (Shedlock and Shen, 2003; Sun et al., 2004). Salmonella-specific CD8 T cells can lyse infected host cells (Lo et al., 1999; Sun et al., 2004) and produce granulysin that has a direct bactericidal effect on salmonella (Stenger et al., 1998; Luu et al., 2006). It was demonstrated that memory CD8 T cells are more important during re-infection (Lo et al., 1999). In addition, both $CD4^+$ and $CD8^+$ cells produce cytokines, necessary for recruitment and activation of phagocytes (Mittrücker et al., 2002). Thus, in our T cell adoptive transfer system, the therapeutic and prophylactic effect could be achieved by the combined action of both $CD4^+$ and $CD8^+$ cell subsets, expressing the transduced single dominant-active salmonella-specific TCRα.

In conclusion, our studies here demonstrated that chain-centric TCRs are ordinarily contained in the normal TCR repertoire, and dominant-active α-chains represent the significant part (20%) of such chain-centric TCRs. Furthermore, we proved that dominant-active TCRα can recognize microbial antigens, and naive T cells, transduced with these TCRα could respond to the specific bacteria both *in vitro* and *in vivo*. These findings are of particular importance as we suggest an approach to identify the therapeutic TCRα, suitable for the adoptive immunotherapy that allows avoiding time - and labor-consuming processes of TCR cloning or amplification of both α- and β-chains TCR from single T cells and controlling of their pairing when transduced into the host T cells. Once identified, therapeutic dominant-active TCRα could be promptly used for transduction of T cells of an MHC-matched recipient in order to generate a large pool of antigen-specific effectors, ready to immediately provide the therapeutic effect.

Furthermore, our experimental approach can open the great perspective for creation of TCRα transgenic animals with innate resistance to certain pathogens without narrowing the diversity of the host TCR repertoire due to the lack of allelic exclusion for α-chain genes in developing T cells.

In studies here, we proved that adoptive immunotherapy based on T cells, transduced with the dominant-specific TCRα could be successfully applied for treatment and prevention of infectious diseases and at the first time represented an example of "vaccination without vaccines" as a perspective alternative to vaccination and existing therapeutic strategies. This approach may be especially effective in the fight against antibiotic-resistant microorganisms, against infections for which there are no vaccines or in a case of a significant risk of side effects after vaccination.

Limitations of the Study

We developed a novel therapeutic approach of adoptive immunotherapy for infectious diseases based on dominant-active antigen-specific α-chains of chain-centric TCRs using salmonellosis in mice as an experimental infectious model. However, considering a vast variety of infections and complex mechanisms of their pathogenesis, further studies using various infectious models are required to prove the efficacy and the universality of the developed therapeutic strategy.

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Dmitry B. Kazansky, kazansky1@yandex.ru.

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The published article includes all data sets generated or analyzed during this study.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101854.

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AUTHOR CONTRIBUTIONS

Conceptualization, D.B.K., L.M.K., and A.A.K.; Methodology, D.B.K., A.A.K., L.N.N., A.V.B., D.M.C., M.I., and O.V.B.; Validation, A.A.K., L.M.K., L.N.N., and D.V.B.; Formal Analysis, A.A.K., L.N.N., and L.M.K.; Investigation, A.A.K., L.N.N., D.V.B., and A.V.B.; Resources, D.B.K., L.M.K., D.M.C., M.I., and O.V.B.; Data Curation, L.M.K, D.B.K.; Writing- Original Draft - A.A.K, A.V.B., D.B.K.; Writing - Review & Editing - A.A.K., L.M.K., and D.B.K.; Visualization, A.A.K. and A.V.B.; Supervision, L.M.K. and D.B.K.; Project Administration, L.M.K. and D.B.K., Funding Acquisition, L.M.K. and D.B.K.

DECLARATION OF INTERESTS

The authors declare no competing interests. The authors have a patent of the Russian Federation, related to this work (Kazansky, D.B., Khromykh, L.M., Kalinina, A.A., Silaeva, Y.Y., Zamkova, M.A., Bruter, A.V., Persiyantseva, N.A., Chikileva, I.O., Jolokhava, L.Kh., Nesterenko, L.N. et al. (2018). A method of creating anti-infectious immunologic defense against Salmonella typhimurium and Listeria monocytogenes by transgenesis of T lymphocytes. Patent №2706554).

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Supplemental Information

Adoptive Immunotherapy Based on Chain-Centric TCRs in Treatment of Infectious Diseases

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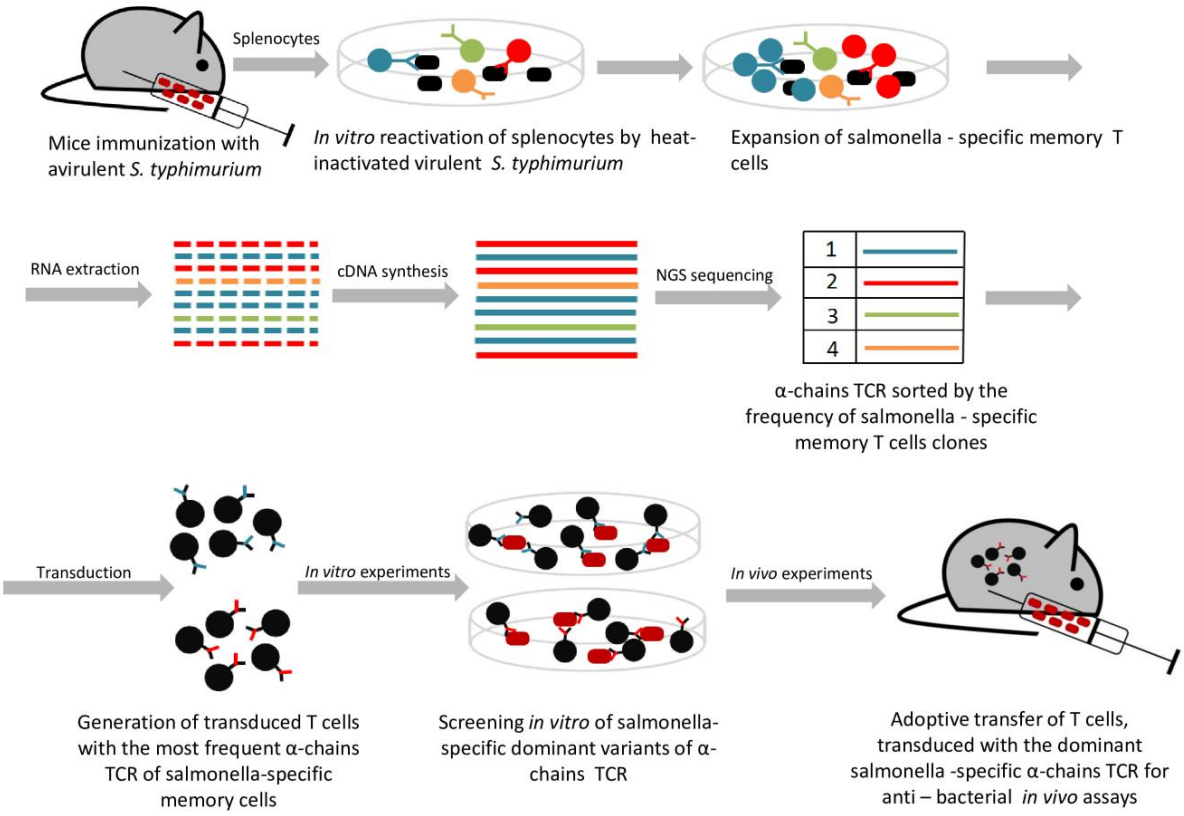

Figure S1. General scheme of experiments. Related to Figure 1, Figure 3, Figure 5, and Figure 6.

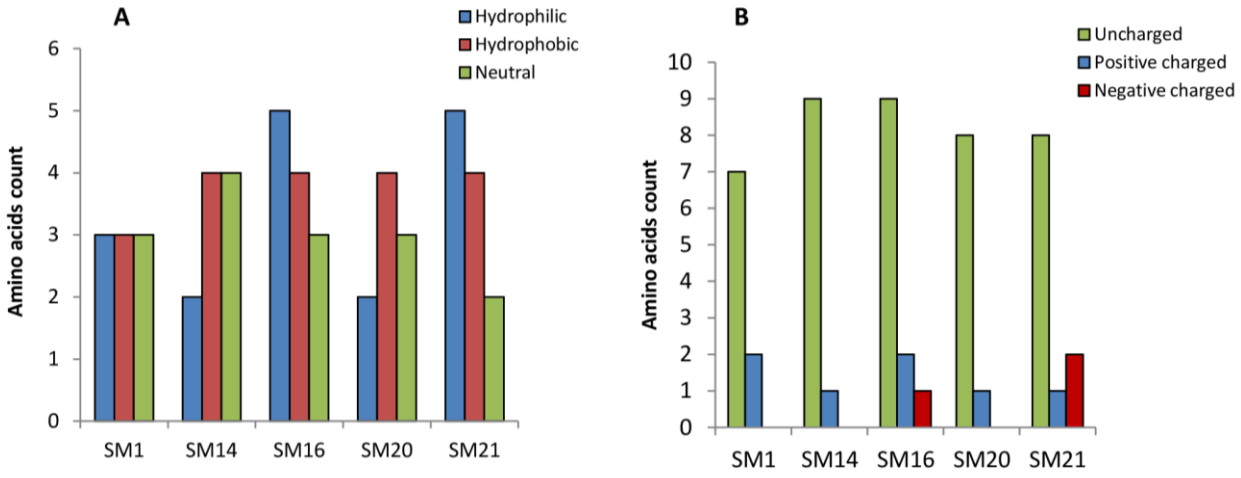

Figure S2. Amino acid (AA) distribution in the CDR3 region of dominant - active salmonella-specific TCRα SM1, SM14, SM16, SM20, and SM21. Related to main-text Results section "Physicochemical properties of the CDR3 region of dominant -active salmonella - specific TCRα**".** Related to Figure 3.
A) Utilization of hydrophobic, hydrophilic, and neutral AA in the CDR3 of each TCRα**. B)** Utilization of uncharged and charged AA in the CDR3 of each TCRα.

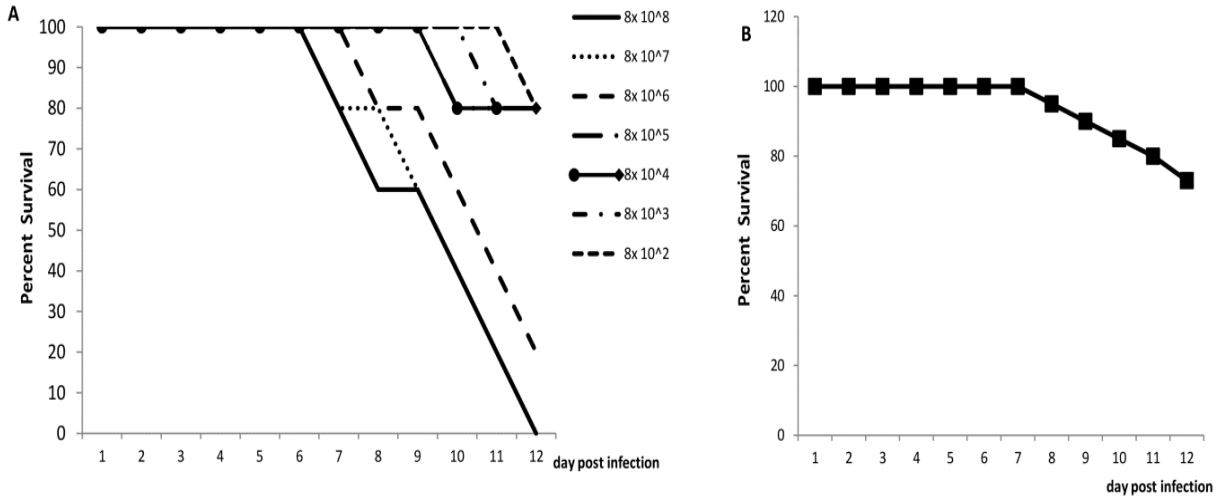

Figure S3. Death dynamics of B10/D2(R101) mice, infected with *S. typhimurium*. Related to Transparent methods. Related to Figure 5, Figure 6.

B10.D2(R101) mice were infected per os via gavage needle with *S. typhimurium* virulent strain IE 147 at doses **(A)** 8.0 x 10^8 - 8.0 x 10^2 CFU/mouse or **(B)** 4.0 - 6.0 x 10^6 CFU/mouse. Animal survival was monitored for 40 days, with animal mortality observed within 8 - 12 days post-infection

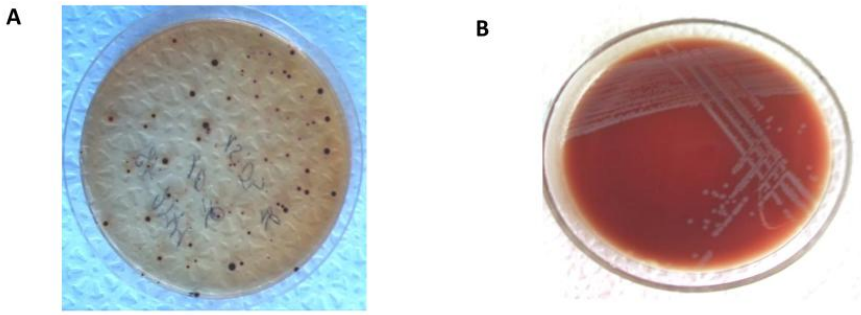

Figure S4. Phenotypic characteristics of *Salmonella typhimurium* virulent strain IE 147. Related to Transparent methods. Related to Figure 1, Figure 5, and Figure 6.
A) Formation of black colonies on SS-agar. **B)** Growth on blood agar without hemolysis.

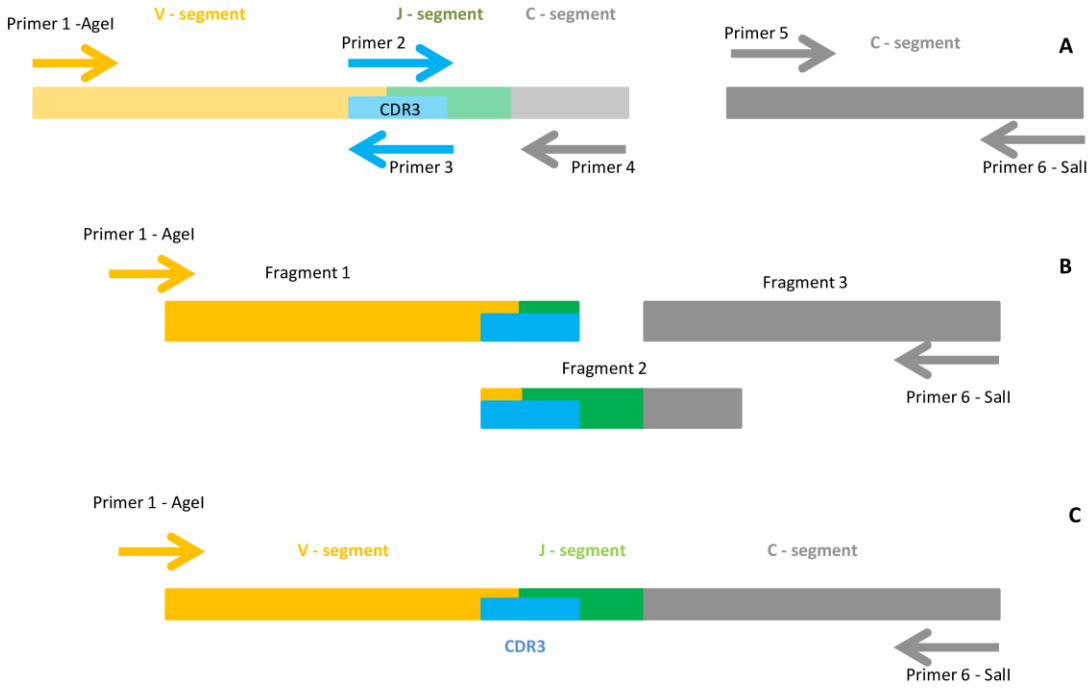

Figure S5. Scheme of selected α-chains TCR cloning. Related to Transparent methods. Related to Figure 3.
A) Schematic presentation of TCRα V-, J-, and C-segments and primers used in cloning. **B)** Three fragments were individually amplified using the combination of Primer 1 – AgeI + Primer 3 (Fragment 1), Primer 2 + Primer 4 (Fragment 2), and Primer 5 + Primer 6-SalI (Fragment 3). **C)** The full ORF of TCRα was amplified by the overlapping PCR using the combination of Primer 1 –AgeI + Primer 6- SalI.

Table S1. Physicochemical properties of the amino acids (AA) in the CDR3 region of dominant -active salmonella - specific TCRα. Related to main-text Results section "Physicochemical properties of the CDR3 region of dominant - active salmonella - specific TCRα"**.** Related to Table 1 and Figure 3.

TCRα	CDR3α full sequence	Length, aa	**Central 5 AA (cCDR3α)**		
			Sequence*	GRAVY**	Normalized polarity
SM1	RVAGYNKLT	9	VAG**Y**N	0,16	0,38
SM14	SAWGNTGKLI	10	**W**GNTG	-1,18	0,28
SM16	REINQGGSAKLI	12	NQGGS	-1,72	0,37
SM20	ITASLGKLQ	9	TAS**L**G	0,74	0,31
SM21	REVMDSNYQLI	11	V**M**DSN	-0,34	0,47

* strongly interacting AA is indicated in red bold
**GRAVY - grand average of hydropathicity index

Table S2. Vα family of the selected dominant -specific α-chains TCR as determined by the amino acid sequence. Related to Table 1.

Variant of α-chain TCR	Vα family
SM1	Va2
SM14	Va3
SM16	Va16
SM20	Va5
SM21	Va16

Supplemental Experimental Procedures

Animals. Mice of inbred line B10.D2(R101) were obtained from the breeding facility of the Federal State Budgetary Institution «N.N. Blokhin National Medical Research Center of Oncology» of the Ministry of Health of the Russian Federation (N.N. Blokhin NMRCO, Moscow, Russia). Mice of inbred line I/StSnEgYCit (I/St), susceptible to *S. typhimurium (*Nesterenko *et al., 2006)*, were obtained from the breeding facility of "N.F. Gamaleya National Research Center of Epidemiology and Microbiology", the Ministry of Health of the Russian Federation ("N.F. Gamaleya NRCEM", Moscow, Russia). Mice were maintained in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments of N.N. Blokhin NMRCO and "N.F. Gamaleya NRCEM". Both female and male mice (18-22 g, 6-8-wk-of-age) were used in the experiments here.

Bacterial strains and growth conditions. *Salmonella typhimurium* virulent strain IE 147 was obtained from H. Tschape from the Robert Koch Institute branch at Wernigerode, Germany. Strain 274, resistant to rifampicin, was isolated, identified and patented in the "N.F. Gamaleya NRCEM". Rifampicin resistance of strain 274 is due to a mutation in the gene encoding RNA polymerase, which leads to a significant decrease in virulence in this strain. Both bacterial strains exhibited characteristic features of *S. typhimurium*, i.e. formed black colonies on SS-agar (Condalab, Madrid, Spain) and grew on blood agar (Condalab) without hemolysis (Figure S4). Bacterial strains were grown overnight in LB broth (AMRESCO, Inc. Solon, OH). To assess the bacteria growth, 10-fold serial dilutions of the cultures were seeded on SS agar. The numbers of colonies were assessed as described elsewhere. To confirm the avirulence of the strain 247, susceptible I/St mice were i.p. injected with 4.0 $x10^6$ CFU (a lethal dose for virulent strains). No animal mortality was detected during 1 month of observation. Freshly grown bacterial strains IE 147 and 247 were used for infection or immunization of B10.D2(R101) mice, respectively. Bacterial cells of virulent strain IE 147 were heat - inactivated (1h, 60ºC) and used in *in vitro* tests.

Infection of mice. To determine LD50 of *S. typhimurium,* B10.D2(R101) mice were infected per os via a gavage needle with the virulent strain IE 147 at doses 8.0 x 10^2 - 8.0 x 10^8 CFU/mouse in 200 µl sterile saline (Figure S3A). The theoretical LD50 was calculated according to Kerber and amounted 1.0 x 10^7 CFU/mouse. For *in vivo* adoptive transfer experiments here, B10.D2(R101) mice were similarly infected with 4.0 - 6.0 x 10^6 CFU/mouse of the virulent strain IE 147 that corresponded to LD27 with animal mortality within 8 - 12 days post-infection (Figure S3B).

Immunization of mice. To generate salmonella-specific memory T cells, B10.D2(R101) mice were i.p. immunized with 1.0 $x10^6$ CFU/mouse of avirulent strain 247 in 500 µl sterile saline. Immunized mice were used in *in vitro* tests on Day 21 post-immunization. Intact (non-immunized) mice were used as the background control.

Isolation of spleen cells. Immunized and intact mice were sacrificed by cervical dislocation, spleens were aseptically isolated and homogenized in a Potter homogenizer in 3 ml of sterile PBS. Cell suspensions were centrifuged (200 g, 5 min, 4^0C) and resuspended in 3 ml of RPMI-1640 medium (PanEco, Moscow, Russia). Viable spleen cells were counted after trypan blue - eosin staining.

***In vitro* enrichment of salmonella - specific memory T cells.** 3.0 x 10^5 splenocytes of B10.D2(R101) mice, immunized with avirulent strain 247, were cultured with 2.0 x 10^3 - 2.0 x 10^7 CFU of heat-inactivated virulent strain IE 147 in 200 µl of RPMI-1640 medium (PanEco), supplemented with 10% fetal bovine serum (HyClone, GE Healthcare, Chicago, IL), 0.01 mg/ml ciprofloxacin (KRKA, Novo Mesto, Slovenia), 0.01 M HEPES (PanEco), and 10 mM 2-mercaptoethanol (Merck, Darmstadt, Germany) (complete medium, CM) at 37ºC, 5% CO_2 for 72 h. Cell proliferation was measured by incorporation of ^{3}H- thymidine (Saint-Petersburg 'Izotop', Saint-Petersburg, Russia), added for the last 16-18 h of culture. The level of the proliferative response of intact (non-immunized) B10.D2(R101) mice was used as the control. For two rounds of *in vitro* restimulation, spleen cells of immunized and intact mice were similarly cultured with 2.0 x 10^4 - 2.0 x 10^6 CFU of heat-inactivated virulent strain IE 147 for 72 h, then 2.0 x 10^5 CFU were added in 10 µl of CM and cells were cultured for another 72 h at 37ºC, 5% CO_2.

Flow cytometry analysis. Cell samples (5.0 x 10^5 cells) were incubated with Fc block (clone 2.4G2, BD Pharmingen) (10 min, 4ºC) and then stained with the following antibodies (40 min, 4ºC): Brilliant Violet 421 - conjugated anti-CD3 (clone 145-2C11, BioLegend, San Diego, CA); Pacific Blue-conjugated anti-CD8a (clone 53-6.7, eBioscience, San Diego, CA), PE-conjugated anti-CD4 (clone GK 1.5, BioLegend). The analysis was performed on FACS CantoII flow cytometer (BD, San Jose, CA) using the FACSDiva 6.0 program (BD). Leukocytes were gated based on the parameters of side (SSC-A) and forward (FSC-A) scatter followed by gating of singlets based on FSC-H vs. FSC-A parameters. Dead cells were excluded from the analysis by propidium iodide staining (PI, BD Bioscience). Further analyses of surface markers expression were performed in the population of live singlet leukocytes. The results were analyzed using Flow Jo 7.6. (TreeStar Inc., Ashland, OR).

Generation of cDNA libraries. Cell suspensions were prepared as described above from spleens harvested individually from two B10.D2(R101) mice, immunized with avirulent strain 247 as described above. The splenocytes were cultured *in vitro* with two- rounds of re-stimulation by salmonella using 2.0 x 10^6 and 2.0 x 10^5 CFU of heat-inactivated *S. typhimurium* virulent strain IE 147 for the first (72 h) and the second (72 h) re-stimulation, respectively. After two-rounds of *in vitro* re-stimulation by salmonella (6 days *in vitro* cultivation in total) 5.0 x 10^5 - 1.0 x 10^6 spleen cells of

each mouse were used for RNA isolation using TRI reagent (MRC, Inc., Cincinnati, OH). In parallel, splenocytes of these two immunized B10.D2(R101) mice, similarly cultivated for 6 days without the antigen stimulation were harvested for RNA extraction. Using all amount of isolated RNA, cDNA libraries of TCRα of each mouse were prepared as described earlier (Egorov *et al., 2015)*. After two-rounds of PCR amplification as described in ref (Egorov *et al., 2015),* the samples were purified and true-seq adapters were ligated according to the manufacturer`s recommendations (Illumina, San Diego, CA). Next-generation sequencing was performed on MiSeq platform (Illumina) using the Myseq reagent kit (300 cycles). Raw data was processed using MiGEC (Shugay et al., 2014). Briefly, a total of 2,194606 raw reads were obtained which contained 438,151 unique molecular identifiers (UMIs). The data was processed with the threshold 6 reads per UMI for all samples. CDR3-containing reads labeled with identical UMIs were assembled into a single molecular identifier group. Further clonotype extraction from the MiGEC-assembled data was performed using MiXCR software (Bolotin et al., 2015). As a result, two cDNA libraries were generated for each immunized B10.D2(R101) mouse that contained TCRα clonotypes with or without *in vitro* re-stimulation by salmonella. Next, two cDNA libraries (with and without re-stimulation) of each mouse were compared, and clones with at least 1.5-fold increased frequency after re-stimulation were selected for subsequent TCRα cloning.

Cloning of TCRα. Using cDNA generated for NGS-sequencing as the template, V-, J-, and C-segments of 23 selected variants of TCRα were amplified. For this, several oligonucleotides were used: three primers within C-segment, common for all TCRα; one primer to the 5'-end of the ORF, generally unique for different α-chains but common for several α-chains; and two primers (forward and reverse) strictly unique for the CDR3 of each TCRα (Figure S5; Table S3). The resulted amplified V-, J-, and C-segments had the pair wise overlap (Figure S5B), and to obtain the full ORF of each TCRα PCR was performed with 5'-V (Primer 1 -AgeI) and 3'-C (Primer 6 - SalI) primers using the mixture of respective three segments as the template (Figure S5C; Table S3). AgeI and SalI restriction sites were additionally introduced into the 5'-V and 3'-C primers, respectively, to clone the full ORF of each TCRα into the MigRI expression vector. All primers used in TCRα cloning are listed in the Table S3. All cloned 23 variants of TCRα were sequenced and compared with the predicted sequences. No mismatches were found.

Transfection. The full-length cDNA of α-chain TCR or GFP were cloned into the MigRI retroviral vector under the PGK-promoter. pCL-Eco plasmid, kindly provided by Beliavskiĭ A.V. (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia) was used as a packaging plasmid for retroviruses. The 293T cell line was transfected with the plasmids by the calcium-phosphate method.

Transduction. Splenocytes of B10.D2(R101) mice were isolated as described above and activated with 3.0 μg/ml concanavalin A, a T cell mitogen and activator (Ando et al., 2014) (ConA, Sigma Aldrich, St. Louis, MO) and 10.0 U/ml murine interleukin-2 (IL-2, BioLegend) for 24h (Kazansky et al., 2018). Retroviral transduction of activated lymphocytes was performed by two rounds of spinoculation (2000 g, 2 h, 22ºC) with viruses collected 48h and 72h post-transfection for the first and the second spinoculation, respectively (Kazansky et al., 2018). 2 - 4 h post-second spinoculation cells were washed in CM (200 g, 5 min, 4ºC) to remove redundant viruses and then cultured in CM supplemented with 10.0 U/ml IL-2 for 72 h (Kazansky et al., 2018). The efficiency of transduction was determined on Day 3 post-transduction using flow cytometry by staining with APC-conjugated anti-Vα2 antibodies (clone B20.1, BioLegend) to evaluate the expression of corresponding transduced TCRα (e.g., SM1) or by measuring GFP fluorescence in parallel control probes transduced with GFP to evaluate the expression of transduced TCRα that belong to Vα families, for which no commercial antibodies were available. The transduction efficiency amounted 40 - 70%.

Preparation of antigen - presenting cells (APC). We used a mix of the syngenic macrophages feeder layer and syngenic splenocytes as antigen-presenting cells. To generate the macrophages feeder layer, B10.D2(R101) mice were i.p. injected with 3 ml of 3.0% sterile peptone (Sigma Aldrich), and 3 days later macrophages were harvested from the peritoneal cavity in 3 ml of ice-cold PBS. 1.0×10^4 macrophages were cultured with 1.0×10^5 CFU of heat-inactivated virulent strain IE 147 in 200 μl of CM per well of flat-bottom 96-well plates (Corning Costar, Sigma Aldrich, St. Louis, MO) for 72 h at 37°C, 5% CO_2. Then macrophages were washed 2 times with pre-warmed CM (37°C) to remove bacterial cells. In parallel, control macrophages were similarly cultured without bacterial cells to generate the unloaded feeder layer. 5.0×10^5 syngenic intact splenocytes were treated with mitomycin C (25 μg/ml, 30 min, 37°C, Kyowa Hakko Kogyo Co., Ltd., Japan) to prevent cells proliferation, washed twice in CM (200 g, 5 min, 4ºC) and then added to the resulted unloaded macrophages feeder layer (unloaded APC) or the macrophages feeder layer, loaded with salmonella (loaded APC) in 100 μl of CM.

TCRα *in vitro* screening. T cells, transduced with TCRα or GFP, were added in the amount of 3.0×10^5 cells to loaded APC in 100 μl of CM and cultured for 72 h. To estimate the background proliferation, transduced T cells were similarly cultured with unloaded APC. Transduced T cells were seeded in three technical repeats. Cell proliferation was measured by ^{3}H-thymidine incorporation as described above. To determine the level of the antigen-induced response of transduced T cells, the level of the background cell proliferation was subtracted from the level of cell proliferation in the presence of loaded APC. The level of antigen-induced proliferation of non-transduced cells (NTC) was used as the reference. The antigen - induced proliferation of TCRα - transduced T lymphocytes at least 2.0-fold exceeding the reference level was considered as an indication of the dominant active α-chain that formed chain-centric TCRs after pairing with random endogenous α-chains of T lymphocytes.

Analyses of physicochemical properties of CDR3 regions of dominant-active TCRα. Evaluation of physicochemical properties of amino acids (AA) in the CDR3 region of 5 functional dominant - active salmonella - specific TCRα chains (SM1, SM14, SM16, SM20, and SM21) were performed using VDJ tools (Shugay et al., 2015). All physicochemical properties of each AA were obtained from the IMGT database (Lefranc et al., 1999). The first 3 and the last AA were removed from the analyses of the CDR3α sequence (obtained from the generated cDNA libraries, Table 1) as described previously (Wang et al., 2012; Yu et al. 2019), and AAs distribution was analyzed in the resulted CDR3α sequence (Table S1, Fig. S2). Averaged values of strength, hydropathicity, and polarity were calculated for the central 5 AAs of the CDR3α of each TCRα.

Adoptive transfer. T cells, transduced with the salmonella-specific TCRα were i.v. injected to B10.D2(R101) mice in a dose 3.0 x 10^5 cells/mouse in 200 μl of PBS 3 days before or 3 days after infection with virulent strain IE 147 for analysis of their prophylactic or therapeutic activity, respectively. NTC were similarly transferred to animals as the negative control.

Analysis of bacterial loads. On Day 7 post - infection spleens of mice were aseptically isolated and homogenized in a SilentCrasher M homogenizer (Heidolph, *Schwabach, Germany)* in 1 ml of sterile saline. To estimate salmonella loads, serial dilutions of spleen homogenates were plated onto SS-agar. Colonies were counted following 12 h incubation at 37 º C.

Statistical analysis. Data are presented as mean ± SD. All statistical analyses were performed using the unpaired Student`s t-test and one-way analysis of variance (ANOVA). A p-value < 0.05 was considered significant. All statistical analyses were performed using Prism software (v. 8.1.2, GraphPad, San Diego, CA).

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