Role of anti-CD3 in modulation of Th1-type immune response in *Shigella dysenteriae* infection

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A murine model was used to evaluate the role of anti-CD3 in modulating a Th1-type response by restimulation of T-cells after immunization with the 57 kDa immunodominant antigen of *Shigella dysenteriae* 1 outer-membrane proteins (OMPs), followed by *Shigella* infection after immunization. To observe the effect of anti-CD3, other T-cell cultures were also established following anti-CD1, anti-IL2 and phytohaemagglutinin stimulation. Anti-CD3 stimulation of reconstituted T-cells showed ‘mean’ levels of CD4 and CD25 were enhanced by 34.5 and 31.1 % in immunized mice, which was comparable to 53.2 and 50.7 %, respectively, in challenged-immunized mice, and were dominant over CD8+ T-cells. Levels of IL2 generated by anti-CD3-stimulated T-cells of immunized mice were greater than those of unstimulated T-cells and were significantly elevated in challenged-immunized mice. The reactivity of T-cells indicated their complete responsiveness, as anti-CD3 antibody might not inhibit the migration of the macrophages but rather inhibit IL4. These macrophage factors synergistically act with anions towards an activated response, which in turn provokes IL2 secretion with a low degree of internalization of its receptor. Thus, sharing of IL2 to form a high-affinity receptor complex with CD4+ T-cells through motive signals suggested a generalized T-cell activation with increased humoral responses. Macrophage migration inhibition factor (MIF) and IL4 responses during anti-CD3 stimulation of immunized mice indicated that the role of anti-CD3 in generation of O2 is due to a synergistic effect by Th1 subsets of Th0 cells. The above findings should have implications for understanding the immunoregulatory role of anti-CD3 associated with 57 kDa antigen in immunoprophylactic measures.

INTRODUCTION

In shigellosis, immunity to *Shigella* infection is mediated mainly by sensitized T-cells (Acha-Orbea, 1993; Herman et al., 1991). This has been well documented by inhibition of delayed type hypersensitivity, immuno-proliferative responses and in cytokine functions (Sinha et al., 1994; Azim et al., 1996; Raqib et al., 1995). The protective immunity often appears late, as a result of which patients may develop some clinical manifestations (Bhattacharya et al., 1988). The immunological basis of *Shigella* destruction has been reported earlier (Way et al., 1998) and the cellular basis of suppression in *Shigella* patients during acute infection is still not yet clear. However, recent studies on Th1 and Th2 subsets suggest multiple causes of T-cell suppression, including biased induction of a Th2 subset (Clement, 1992). The invasive mode of *Shigella* infection and the undermining of its protective Th1 response has been described mostly in experimental studies and profound T-cell suppression was observed (Borisova, 1999). This demands an alternative to our existing concept of the cell-mediated immunity pattern that exists in shigellosis. However, CD4+ T-cell activation is associated with the expression of CD25 (IL2 receptor (IL2R)) for interleukin-2 (IL2) release and other macrophage inhibitory factors, which are required for T-cell regulation during the acute stage of *Shigella* infection (Rose-John & Heinrich, 1994). The presence of HLA-DR antigen together with CD4+ T-cells in the gut mucosa of acute patients (Raqib et al., 1994) signifies the possible role of Th1 responses in protection against shigellosis.

A direct effect of *Shigella* alone could not explain their immune stimulating moieties. The absence of productive T-cells during infection might be due to their poor early activation. Therefore, the regulatory activities of memory T-cells need to be considered. Anti-CD3 identified on T-cell subsets has been known to exhibit receptor-like properties in significantly proliferating memory T-cells, inducing the cytotoxic activity of natural killer cells (Maluccio et al., 2001) and enabling cytokine release through directed signals (Yanagida et al., 1994). Memory T-cells play an important role in mediating antigen recall response for the helper cells to acquire memory and specificity towards bacteria. In

Abbreviations: APC, antigen presenting cell; MIF, macrophage migration inhibition factor; PHA, phytohaemagglutinin.

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addition, memory T-cells have been shown to demonstrate significant proliferation with interferon gamma (IFN-γ) production, which strongly reflects their role in regulation of Th1 cytokines. Anti-CD3 stimulation of murine T-cells has been shown to produce IL2-induced T-cell activation (Tamura et al., 1993). During T-cell activation, phosphorylation is switched on and oxygen free radicals are generated spontaneously (Cantrell et al., 1987). As a result, anti-CD3 stimulation up-regulates protein-kinase-C-mediated phosphorylation in the CD4+ T-cells (Matsuyama et al., 1991), which in turn leads to IL2 production rather than IL4 (Nguyen et al., 1995), superoxide ion (O2-) formation (Kaur et al., 1998) and IL2R (CD25) expression on macrophages with class 1 molecules (Brams & Claesson, 1989). However, its physiological role in influencing helper T-cells is yet to be ascertained in shigellosis.

The susceptibility to Shigella infection following immunization with LPS or outer-membrane proteins (OMP) of S. dysenteriae 1 may be due to lack of antigen reactive T-cells and leads to immuno-suppression in shigellosis (Borisova, 1999; Czarny et al., 1992). Earlier, we have reported the consistency in antigenic recognition of a 57 kDa major antigenic fraction of OMPs by acute and convalescent sera of Shigella-infected patients (Sinha & Chakrabarti, 1992). Furthermore, the role of this antigen has been demonstrated in adhesion to HeLa cells and in the production of IL2 by induction of the CTLL-2 cell line (Chakrabarti & Sinha, 1994, 1997). Hence, the present study was carried out to evaluate the in vitro T-cell responses in immunized BALB/c mice using the 57 kDa major antigenic fraction, followed by restimulation with anti-CD3 mAb and other reagents [phyltohaemagglutinin (PHA), anti-CD1 and anti-IL2]. This may suggest a role for anti-CD3 in enhancing resistance to infection by way of increasing IL2 and phenotypic expression of IL2R on memory T-cells. Furthermore, it should help to evaluate the effect of restimulation on challenged-immunized mice with a lethal dose of homologous species. Reduced IL4 responses were observed and indicated that an exposure of anti-CD3 to T-cells can reconstitute the antigen reactive B- and T-cell responses in the context of resistance against shigellosis.

**METHODS**

**Bacterial culture and preparation of 57 kDa OMP.** Serovar-specific strains of S. dysenteriae type 1 were isolated from the faces of patients with bacillary dysentery admitted to Paediatric and General wards of the Infectious Diseases Hospital, Kolkata. Bacteriological examination of stool was done by standard techniques (WHO, 1993). The strain (PB10) was identified and confirmed by using API 20E biochemical test (bioMérieux) and different antisera A (Difco). After guinea pig passage as described by Sereny (1957), virulent smooth colonies were grown at 37 °C in tryptic soy broth (TSB; Difco). OMPs were isolated by using a standard method (Johnston & Gotschlich, 1974). In brief, after harvesting the cells from culture, they were sonicated and treated with Sarkosyl (sodium lauryl sarcosinate, 1%, w/v; Sigma) for 30 min at 24 °C, to selectively solubilize the inner membrane, followed by centrifugation at 100,000 g for 2 h. The pellet containing OMPs was washed with distilled water and stored at −20 °C. The major antigenic fraction (57 kDa) was eluted from gel slices electrophoretically using an electro-eluter (Bio-Rad) as described previously (Sinha et al., 1994). The protein was concentrated using a Speed-Vac (Savant SC-210A) and the concentration was measured using 1% BSA as standard, as described by Markwell et al. (1978). To ascertain whether the eluted protein contained trace amounts of bound LPS, the Limulus amoebocyte lysate (Sigma) assay was performed using Escherichia coli O55:B5 LPS (Sigma) as control (Yin et al., 1972).

**Immunization and challenge.** Inbred male 3–4-month-old BALB/c mice were housed in the animal unit of this institute according to institutional guidelines. They were grouped into (i) immunized, (ii) infection after immunization and (iii) control groups, each containing five mice. The first two groups were immunized subcutaneously with 25 μg of the 57 kDa antigen emulsified in Freund’s adjuvant at the 1st, 2nd and 3rd week, followed by a booster dose of 50 μg of the 57 kDa antigen at the 5th week. After 10 days, animals were kept on glucose-water feeding and were challenged with a lethal dose of 1 × 108 c.f.u. ml⁻¹ bacteria (S. dysenteriae type 1; strain PB10) orally (Mallet et al., 1995). The challenged mice were monitored for a month (one mouse died 24 h after challenge).

**Specific antibody response against immunization.** To evaluate the induced immunity in immunized mice, the kinetics of the humoral IgG response was determined by ELISA (Voller et al., 1978) on days 3, 10, 21 and 28 in immunized mice with respect to day 0 for the control group. The same response was also observed in challenged-immunized mice at days 1, 14, 21 and 28. The serum antibody responses were measured as the inverse log [ln (x)] of the titre value measured at 492 nm.

**Isolation, enrichment of T-cells and in vitro stimulation of macrophages.** Spleen and lymph node cells (from two mice of each group) were isolated by homogenizing the tissues in cold PBS followed by centrifugation at 400 g for 10 min at 4 °C. The cells were washed twice in supplemented RPMI 1640 medium (Sigma). On average, more than 90% of the cells were viable using the trypan blue exclusion method. Finally, 1 × 10⁶ cells ml⁻¹ were suspended in complete RPMI medium supplemented with 10% FBS and 100 U gentamicin ml⁻¹. Later, T-cells were fractionated from the total cell population using a panning method (Payne et al., 1981). Briefly, total cells were incubated for 2 h at 37 °C and 5% CO₂ in 6-well tissue culture plates (Corning) with glass coverslips, then the supernatants containing T- and B-cells were placed in anti-Ig-coated Petri dishes for 1 h at room temperature. Non-adherent T-cells were separated from B-cells adherent to anti-Ig and washed once with complete RPMI 1640 medium. The cells were then stimulated in duplicate with 5 μg of different stimulants including PHA, anti-CD1, anti-CD3 (Pharmaningen) and anti-IL2 (Sigma) for 48 h at 37 °C in 5% CO₂. Then, the cells were co-cultured with adhered macrophages under the same culture conditions for 96 h.

**Avidin–biotin complex-immunoperoxidase staining using specific monoclonal antibodies.** Stimulated or unstimulated cells were allowed to adhere to 22 mm glass coverslips in 6-well tissue culture plates in complete RPMI 1640 medium supplemented with 10% FBS in 12 mL Hepes and 50 U gentamicin ml⁻¹ at 37 °C in 5% CO₂. Coverslips were washed with RPMI medium and once with PBS with TWEEN-20 (PBS-T) and 1% BSA to remove non-adherent cells, then fixed with 0.25% glutaraldehyde. The cells were then incubated with biotinylated monoclonal antibodies to CD4, CD8 and CD25 individually, in duplicate for 30 min at 37 °C. After washing with PBS-T containing 1% BSA, avidin peroxidase (1:100 in PBS) was added. Subsequently, the cells were stained with di-aminobenzidine (DAB, 0.5 mg ml⁻¹) followed by 0.1% haematoxylin to observe percentage marker expression on the cell surface, quantified by using automated video microscopic analysis (Olympus) in a 100 μm² area (Raqib et al., 2002).
Macrophage migration inhibition assay. Macrophage migration inhibition factor (MIF) generation before and after infection of immunized mice was evaluated in a macrophage agarose assay (Clausen, 1971) and the values were compared with baseline data (in cells only) as well as from corresponding values obtained from other study groups (stimulated cells). Briefly, 3 mm diameter wells were cut into agarose (1-3 %) for a reaction of lymphocytes with different stimulants. After 24 h of incubation at 5 % CO2 at 37 °C, reactivity of T-cells was assessed by measuring % macrophage MIF (Bloom & David, 1973) after fixation with 0-37 % formaldehyde and staining with Giemsa stain.

ELISA for IL2 and IL4. The assay was as described in the WHO manual (WHO, 1999). In brief, 96-well microplates (Nunc) coated with 0-5 μg captured antibody per well in PBS (pH 7-2) were incubated overnight at 4 °C in the refrigerator. Next day, the plates were washed twice with washing buffer (PBS-T with 1 % BSA) at room temperature. The non-incubated cultures (described earlier), in 30 min at 37 °C and washed buffer (PBS-T with 1 % BSA) at room temperature. The non-specific sites were then blocked with 5 % BSA (IgG-free) in PBS for 30 min at 37 °C. The plates were washed thrice with washing buffer and incubated for 2 h at 37 °C with different serial dilutions (twofold) of standard and differently stimulated cultures (described earlier), in duplicate, in PBS. The wells were again washed thrice with washing buffer and incubated with 1 μg biotinylated antibody ml-1 in PBS. Unbound antibody was removed by washing thrice with washing buffer. One hundred microlitres of a 1: 2000 dilution of streptavidin–horseradish peroxidase conjugate was added to each well. After washing three times, the colour was developed after adding 50 μl per well of 0-1 % substrate [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] in 0-1 M sodium citrate buffer (pH 4-5) and 0-1 % H2O2 for 15 min at 37 °C in the dark. Finally, the reaction was stopped by adding 20 μl 10 % SDS. The absorbance was recorded at 492 nm in an ELISA reader (Bio-Rad). Calculations were performed to estimate the concentrations of IL2 and IL4 levels in the culture supernatant.

Superoxide dismutase (SOD) assay. Macrophage cells (104 cells ml-1) with different stimulation-primed T-cells were mixed with 50 μM cytochrome c (Sigma) in 0-5 ml Hank’s balanced salt solution (HBSS) (Roiilides et al., 1990). The mixture was incubated with 0-5 μM phorbol myristate acetate (PMA; Sigma) for 15 min at 37 °C in a shaking water bath. Reactions were performed in duplicate against identical control wells containing 20 μg SOD ml-1 (Sigma) with respect to controls (without any cells). O2 production was assessed as the difference in absorbance at 570 nm compared with control and was calculated using the extinction coefficient of 21-1 × 103 M/cm for reduced cytochrome c as described by Green et al. (1998).

Statistical analysis. Two-way analysis of variance and Student’s t-test were performed to compare the effect of stimulants (in duplicate) for each variable in the immunized and infection after immunization groups of mice. The data for immunoassays were processed using a software package (Epistat) to generate a curve using linear regression analysis and expressed as mean ± SE for three consecutive experiments.

RESULTS

Kinetics of IgG antibody response to immunized and challenged-immunized BALB/c mice at different periods

The antibody response was found to increase on day 3 after immunization and remained significantly elevated until day 10. Despite a significant sixfold decrease in specific IgG level after day 28 (P < 0-001), the baseline antibody level was still notable (not shown). The serum IgG antibody response in challenged-immunized mice changed in a similar fashion to immunized mice. In the challenged-immunized mice the IgG response was significantly increased until day 14 (P < 0-002), followed by a decline on day 21 (P < 0-02). As with the immunized group, this response remained elevated until day 28 of infection after immunization and above the baseline before infection, despite this sharp decline.

Expression of CD4, CD8 and CD25 in differently stimulated T-cells in the presence of antigen presenting cells (APC)

Phenotypic expression of CD4 and CD25 in differently stimulated culture supernatants of T-cells in the presence of APC from immunized and infection after immunization groups were evaluated by the avidin–biotin complex-immunoperoxidase method. It was found that there were 39-0 % CD4- and 32-2 % CD25-positive cells in anti-CD3 stimulated T-cells at day 7 of immunization, which was comparatively higher than at 2 weeks after immunization or in the controls. There was no such significance when compared with other stimulated groups [anti-CD1 (P < 0-05); anti-IL2 (P < 0-01) and PHA (P < 0-05)], with fluctuations in both groups (Table 1). After 1 day of infection in the immunized mice, the expressions of CD4 and CD25 were 57-0 and 51-0 %, respectively, and significant (P < 0-05) proliferation of CD4+ and CD25+ T-cells was found when stimulated with anti-CD3 antibody, relative to the others. These expression levels were maintained until day 21 and found to be significant (P < 0-01 and 0-05) relative to control and unstimulated groups.

Expression of CD4+ T-cells was found to be dominant over CD8+ T-cells in the anti-CD3-stimulated T-cell population of day-7-immunized mice and in challenged-immunized mice, but was found significantly less in controls (P < 0-01). Other stimulated cultures in all groups of mice were shown to be insignificant and fluctuating when compared all together (Table 1).

Cytokine responses during T-cell activation

T-cell responses were observed in the lymph nodes of the immunized and infection after immunization groups and compared with the control group. On day 7 of immunization, increased MIF (37-26 %), IL4 (14-33 μg ml-1) and IL2 (17-34 μg ml-1) responses were found in T-cells activated with anti-CD3 and were significantly higher (P < 0-01–0-05) when compared with other stimulated T-cells, unstimulated T-cells and controls. Furthermore, the equivalent responses of MIF, IL4 and IL2 were noted 2 weeks after immunization and were found to be significantly higher (P < 0-05) in the anti-CD3-activated T-cells (Figs 1 and 2).

The T-cell responses for MIF, IL4 and IL2 release were also noted in infected-immunized mice and increased levels were measured in supernatants of anti-CD3-activated T-cells after day 1. On day 21 of infection, the MIF and IL4 responses were decreased to 25-1 % and 17-5 μg ml-1, respectively, while IL2 production increased to 33-63 μg ml-1; insignificant re-
Table 1. Phenotypic expression (as a percentage) of CD4, CD8 and CD25 in differently stimulated culture supernatants in immunized, infection after immunized and in control mice

<table>
<thead>
<tr>
<th>Stimulants</th>
<th>CD4</th>
<th>CD8</th>
<th>CD25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7 post-immunization</td>
<td>2 Weeks post-immunization</td>
<td>Day 1 infection after immunization</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>39.0 ± 3.3</td>
<td>29.9 ± 1.6</td>
<td>15.3 ± 6.3</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>10.0 ± 1.0</td>
<td>10.0 ± 0.6</td>
<td>10.0 ± 1.0</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>2.3 ± 1.2</td>
<td>2.3 ± 1.0</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>8.3 ± 1.0</td>
<td>8.3 ± 1.0</td>
<td>8.3 ± 1.0</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>3.8 ± 1.0</td>
<td>3.8 ± 1.0</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>10.1 ± 0.7</td>
<td>10.1 ± 0.7</td>
<td>10.1 ± 0.7</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>5.1 ± 0.5</td>
<td>5.1 ± 0.5</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>9.3 ± 0.2</td>
<td>9.3 ± 0.2</td>
<td>9.3 ± 0.2</td>
</tr>
<tr>
<td>Anti-CD3 Anti-CD1 Anti-IL2 PHA Unstimulated</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
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Fig. 1. Release of MIF at different time periods of immunized and infected APCs co-cultured with differently stimulated and unstimulated T-cell (primed) supernatants as compared to controls.

Fig. 2. Kinetics and pattern of IL2 (bars) and IL4 (circles) at different times from APC of immunized and infection after immunization mice co-cultured with differently stimulated and unstimulated T-cell (primed) supernatants as compared to controls: (a) day 7 of immunized mice; (b) 2 weeks of immunized mice; (c) day 1 of challenged-immunized mice; (d) day 21 of challenged-immunized mice and (e) controls.

Superoxide ion (O2) production during T-cell activation

The levels of superoxide ion were measured in stimulated culture supernatants of immunized, challenged-immunized and control groups of mice. Anion production in anti-CD3-stimulated cultures from immunized mice on day 7 [14.25 nmol (10⁵ cells)⁻¹] and 2 weeks [8.41 nmol (10⁵ cells)⁻¹] was significantly higher (P<0.05) than that of controls [2.5 nmol (10⁵ cells)⁻¹] (Fig. 3), while it was not significant in unstimulated cultures. However, this response was less significant when compared with day 1 [6.01 nmol (10⁵ cells)⁻¹] of infection of the immunized mice, but was

spesses were found in other stimulated culture supernatants (Figs 1 and 2). In control groups of mice, the same response was seen in anti-CD3-stimulated cultures.
Immunity level was already restored through successful (infection after immunization) in which the cell-mediated groups (immunized). This was compared with a group persistence of antibody would help in achieving an increased cell-dependent humoral immune response that leads to IgG production (Islam et al., 1995, 1996). In the present study, the IgG response was seen for a maximum period of 28 days despite a fall in titre by threefold after day 7 post-immunization. During this period, the antibody level was decreased (not shown), but the levels of MIF and IL2 were increased (unstimulated groups in Figs 1 & 2). On the other hand, the increased rate following CD4 and CD25 expression were more significant (P < 0.05) on day 1 while being less in CD8+ T-cells (Table 1). These responses were intact until day 21 and 28 of challenged-immunized groups (data not shown).

**DISCUSSION**

There is no selective activation of Th1 (IL2, IFN-γ) or Th2 (IL4, IL10) subsets during *Shigella* infection. The fate of the patient depends on the signals generated for the selection of CD4 or CD8 T-cells. Recovery from the disease would primarily depend on the development of effective mucosal immune responses. Previously, Islam and colleagues defined the role of bacterial antigens in skewing the T-cell receptor Vβ repertoire towards the Th2 response with increased T-cell-dependent humoral immune response that leads to IgG production (Islam et al., 1995, 1996). In the present study, the IgG response was seen for a maximum period of 28 days despite a fall in titre by threefold after day 7 post-immunization. During this period, the antibody level was decreased (not shown), but the levels of MIF and IL2 were increased (unstimulated groups in Figs 1 & 2). On the other hand, the IgG level of the immunized mice was changed significantly at day 21 of infection and a subsequent decrease in MIF with an increase in IL2 levels was found when compared with the control group of mice. This observation indicates that the persistence of antibody would help in achieving an increased humoral response after infection in the immunized mice.

Anti-CD3 is a ligand of T-cell receptors which significantly activates and proliferates memory T-cells towards the cellular targets through directed signals (Delves, 1994; Yanagida et al., 1994). Further, its effects on T-cell manipulation were examined and found positive for CD4 and CD25 in target groups (immunized). This was compared with a group (infection after immunization) in which the cell-mediated immunity level was already restored through successful immunization. This increase in *Shigella*-specific CD4+ T-cells in challenged-immunized mice was highly significant (P < 0.001) in relation to corresponding cell number as observed in target groups. Among the stimulated and unstimulated cells of both groups, anti-CD3-stimulated cells showed a significant response which was later compared with control groups and found lower by 0.5–1-fold. Anti-CD3 produced a change in cytokine kinetics of the antigen-specific response by generating MIF and IL2 in target groups. Two weeks after immunization, the mice were found to have hyper-reactive T-cells, as anti-CD3 antibody significantly proliferates the memory of T-cells, enabling them to unleash cytokines through motive signals (Kruisbeek, 1991) towards *Shigella*. However, when these cells were exposed to anti-CD3 after infection with a lethal dose of *Shigella*, primary cytokines (MIF), IL4 and IL2 were released in a reciprocal fashion. Although this has been recorded, a significantly (P < 0.03) enhanced level of IL2 was observed, but the levels of MIF and IL4 were non-significantly decreased when compared with the other stimulated cells of immunized groups (Figs 1 and 2). However, these cytokine responses showed the opposite pattern in the controls (Figs 1 and 2). On the other hand, the increased rate following CD4 and CD25 expression were more significant (P < 0.05) on day 1 while being less in CD8+ T-cells (Table 1). These responses were intact until day 21 and 28 of challenged-immunized groups (data not shown).

Superoxide anions are key molecules in the pathogenesis of various infectious diseases, although oxygen radicals and nitric oxide have an antimicrobial effect on bacteria (Akaie, 2001). Moreover, large amounts of anions at an initial phase of immunization and, to a lesser extent, at later phase of challenged-immunization were observed in anti-CD3-stimulated cells as shown in Fig. 3. We found no significant changes in the release of anions among stimulated and unstimulated controls except for a small change in the anti-CD3-stimulated control group. This was insignificant when compared to targeted and comparable groups. A high release of superoxide anions at the initial phase may lead to antigen processing and expression on APCs but later decreases with inhibitory factors and IL4. Not only the reciprocal relationship of anions, but also MIF release, play important roles in IL2 production due to anti-CD3 infusion.

The Freund’s adjuvant used along with the 57 kDa antigen might have helped to trigger the T-cell-dependent humoral immune responses and increase the efficiency of the macrophage processing of the antigen. After restimulation with anti-CD3, the reactivity of the T-cells indicated their complete responsiveness, as it might not inhibit migration of the macrophages towards the activated response synergistically with anions. In turn, it provoked IL2 release but not IL4. In parallel, a high level of CD25 (IL2R) expression until day 28 in challenged groups after successful immunization was considered as a generalized T-cell activation with increased antibody response. As a result, increased IL2R sharing with IL2 to form a high affinity receptor complex promotes cell proliferation for CD4+ T-cells through cytokine-specific signals (Watanabe et al., 1999; Taniguchi & Minami, 1993). Consistent with the previous study, anti-CD3-antibody-stimulated T-cells induced Th1 type cyt-
kines (IL2) produced by naïve T-cells in the murine or human system (Fang et al., 2000; Jenkins et al., 1990). However, our data suggested that anti-CD3 stimulation of the immunized mice might shift the specific immune responses towards the Th1-type pattern by inducing APC to produce IL2 in shigellosis.

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