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# Oral Mucosal Tolerance Versus Systemic Immune Response to *Salmonella typhi* Antigen

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**Abstract:** It was demonstrated that the oral vaccine application of *Salmonella typhi* antigen can activate low antibody agglutinin titer (mean:40±0) comparing with high agglutination titer induced by Intramuscular administration of *Salmonella typhi* antigen (mean 560.0 ± 51.64) as well as anti-*Salmonella typhi* IgG ELIZA shows high mean index value(mean = 0.6957±0.10) comparing with the low index value induced by oral rout were (mean= 0.028±0.014) while anti *Salmonella typhi* IgM ELIZA test show mean index value = 0.6339±0.0385 comparing with low IgM index value (mean= 0.1560±0.070) induced by oral rout (Rsquared 0.7457, t test 3.3. The pro –inflammatory cytokines IL-1α was high in intramuscular rout 217.089±39.78 than its concentration with in oral administrated group (100.4±12.09), IL-12 was about the same concentration both in oral rout and intramuscular rout subsequently (23.607 and 23.17) p value 0.01, R squared (0.3958).However the immune responses were not absolutely absent in the oral administrated group, this reflect the fact that there is a selectivity in taking oral antigens from digestive mucosal surfaces but this immune feature and selectivity theme may vary from antigen to another. In conclusion the recent and ongoing expansion of a new information about the mucosal and systemic immune responses lend a promise to provide the tools needed to exploit the full potential and development of both mucosal and intramuscular vaccines.

**Keywords:** Oral Tolerance, Systemic Immune Response, Intramuscular Rout

## 1. Introduction

Many vaccines and antigens that are given orally or deposited directly on mucosal surface ,will face the same gauntlet of host defenses as do microbial pathogens[1] .Such vaccine and antigens are being diluted in mucosal secretion, captured in mucus gels, attacked by proteases and nucleases and excluded by epithelial barriers[2,3] . Thus the exact dose of mucosal administered antigen that actually crossed the epithelial barrier[3,4], cannot be precisely determined but can only be estimated .Meanwhile mucosal tissues microenvironments are adapted to the presence of foreign antigens, such as microorganisms and their products. As a result, vaccines that consist of soluble macromolecules and protein subunit antigens, which may produce vigorous immune responses if injected into a sterile environment such as muscle, are often ignored when applied onto the mucosal surfaces[1,3,4,5,6]

The development of specific antibody- or T-cell-mediated immunologic responses and the induction of mucosal induced systemic immunologic hypo-responsiveness (oral or mucosal tolerance) depend on complex sets of immunologic events, including the nature of the antigenic stimulation of specialized lymphoid structures in the host, antigen-induced activation of different populations of regulatory T cells (Th1 versus Th2), and the expression of proinflammatory (IL-1, IL12 and immune-regulatory cytokines IL-10.[5,16].

So present study aim to evaluate the immune responses of *S. typhi* antigen both in mucosal and intramuscular primed lapin model.

## 2. Main Body

### *2.1. S. Typhi "O" Vaccine*

Local *S. typhi* isolate characterized by API20 was obtained

from an enteric fever cases[7]. *S. tyhpi*"O" antigen was heat killed bacteria [8].

### *2.2. Rabbits*

Fourteen male New Zeeland white rabbits weighing 1.5 -2 kg were kept and housed individually in wire-rod floored and stainless steel cages each measuring 48x 61x46 cm with collection pan beneath each cage. Food and water were given *ad libitum*. They were grouped into two groups.

### *2.3. S. typhi Immunizations*

Group I received two oral doses of *S. typhi*"O" bacterin (10 IU) one week a part.

Group II received two Intramuscular doses, one week a part. Rabbits of groups I and II were left one week then bled.

### *2.4. Blood Sampling*

Blood were collected through cardiac puncture method using sterile disposable syringes, from each animal in each group. Each sample without anti- coagulant for separation of sera to study agglutination[10], ELIZA IgM and IgG and cytokines, cytokine IL-1 alpha, IL-10 and IL-12 determination was performed as in manufacture instructions.

### *2.5. Antibody Score*

The anti-Salmonella typhi antibody titer was scored as the reciprocal of the highest dilution that gave frank positive agglutination results[10] .S. typhi specific IgM and IgG were scored as an index mean values.

### *2.6. Cytokine Score*

The cytokine responses were estimated as an immuno-enzyme color reactions in terms of picograms per milliliters.

### *2.7. Biometric Analysis*

Biometery and graphs were done using PRISIM software.

## 3. Results and Discussion

*Salmonella typhi* specific agglutinin of intramuscular administrated antigen 560 ± 51.64in the *S. typhi* priming rabbite were of higher mean values than that of mucosallyadmintrated*S. typhi* antigen 40± 0 mean values.

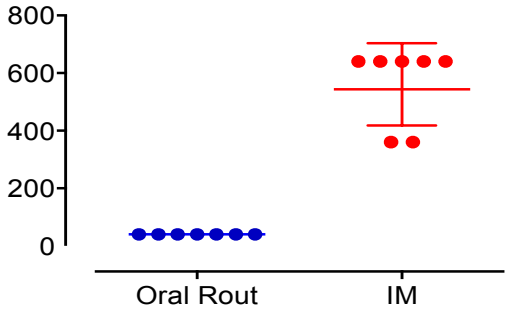
***Table 1.*** *Salmonella specific serum agglutination titers and anti S. typhiIgG and IgM ELISA antibody index values.*

| Test rabbits | Oral Rout | | | Intramuscular Rout | | |
|---|---|---|---|---|---|---|
| | agglutination | IgG | IgM | agglutination | IgG | IgM |
| 1 | 40 | 0.116 | 0.042 | 640 | 0.229 | 0.595 |
| 2 | 40 | 0.017 | 0.164 | 640 | 0.971 | 0.750 |
| 3 | 40 | 0.012 | 0.113 | 360 | 0.980 | 0.755 |
| 4 | 40 | 0.011 | 0.052 | 640 | 0.600 | 0.519 |
| 5 | 40 | 0.013 | 0.084 | 360 | 0.560 | 0.516 |
| 6 | 40 | 0.020 | 0.067 | 640 | 0.960 | 0.601 |
| 7 | 40 | 0.010 | 0.050 | 640 | 0.570 | 0.701 |
| Mean | 40 | 0.028 | 0.08 | 544.4 | 0.668 | 0.627 |

*S. typhi* specific IgM and IgG: The mean index value of IgM and IgG indicate specific for S. typhi in mucosal primed rabbits were(0.028± 0.014) lower than those intramuscular primed rabbits (0.6957± 0.10).

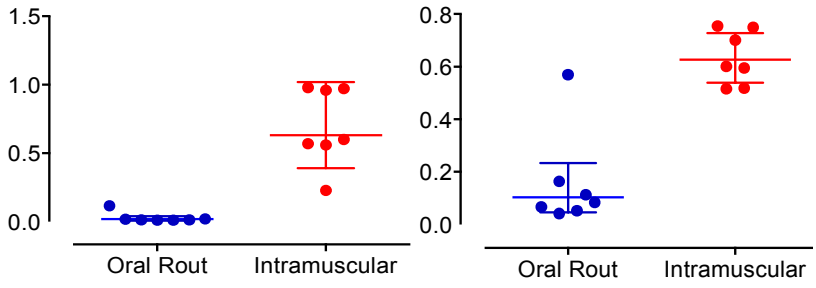
The Cytokine concentration level of *S. typhi* "O" primed rabbits for the cytokine IL-1 αand IL-10 were lower in mucosal than in intramuscular and they were equated for IL-12.(23.607 and 23.17) respectively p=0.01

The oral mucosal immune tolerance were screened by agglutination, IgG, IgM and IL-10. (Table-1, Figure 1-3).




Significantly different (P < 0.05)

***Fig. 1.*** *Agglutination titer for both oral and intramuscular(IM) administrated antigen.*




(A) (B)

Significantly different (P < 0.05)

***Fig. 2.*** *Comparison IgG (A) and Igm (B) ELIZA antibody index values in both Oral and Intramuscular rout.*

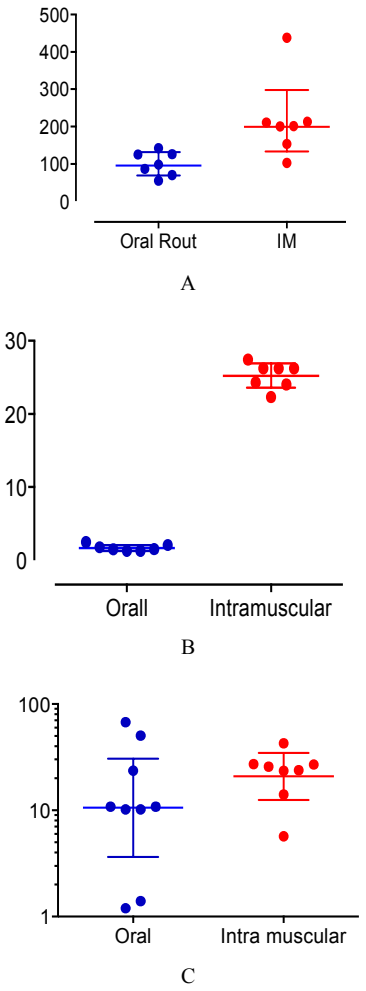
***Table 2.*** *Cytokine profile of S. typhi primed rabbits .*

| Test Rabbits | Cytokine profile pg/ml | | | | | |
|---|---|---|---|---|---|---|
| | IL-1 alpha | | IL-12 | | IL-10 | |
| | Oral | IM | Oral | IM | Oral | IM |
| 1 | 125.98 | 153.549 | 1.2 | 23.45 | 2.1 | 24.0 |
| 2 | 125.08 | 437.667 | 1.4 | 5.7 | 2.5 | 24.3 |
| 3 | 98.42 | 102.36 | 10.2 | 27.005 | 1.8 | 22.3 |
| 4 | 55.11 | 200.14 | 10.8 | 42.788 | 1.3 | 26.2 |
| 5 | 141.73 | 211.11 | 10.8 | 14.03 | 1.3 | 26.2 |
| 6 | 86.61 | 201.20 | 67.58 | 25.8 | 1.5 | 26.2 |
| 7 | 70.01 | 212.84 | 50.43 | 27.2 | 1.53 | 27.4 |
| Mean | 100.4± 12.09 | 217.089± 39.78 | 23.607 | 23.82 | 1.719± 0.16 | 25.23± 0.6636 |
| Control | 15.3 | | 7.32 | | 4.450 | |

***Table 3.*** *Oral Systemic Tolerance in S. typhi primed rabbits.*

| Rout | Agglutinin* | IgG* | IgM* | IL-1 α* | IL-12* | IL-10* |
|---|---|---|---|---|---|---|
| Oral | 40 | 0.028 | 0.08 | 93.277 | 23.607 | 1.846 |
| Intramuscular | 542 | 0.668 | 0.62 | 217.089 | 23.17 | 24.48 |
| Negative Control | 80 | 0.017 | 0.073 | 15.3 | 7.32 | 4.4509 |

*mean values of the immune function tests. IgM, IgG in index values, cytokines in pg/ml.




Significantly different ($P < 0.05$)

***Fig. 3.*** *Cytokines profile of IL-1(A); IL-10 (B) and IL-12 (C) Elisa in both Oral and Intramascular rout.*

The results presented in table 1 and figure 1-3 were showing an endeno for mucosal immune tolerance induction through oral priming immunization program.Such finding may provide an endewe that mucosal exposure to environmental macromolecules, infectious agents and dietary antigens can result in the immunological state of development of systemic hypo- responsiveness toward the inducing antigen [11].Low IL-12 apparently can not be of use as indicator to the development of oral mucosal immune tolerance, other observation improved that systemic administration of Abs to IL-12 (anti-IL-12) simultaneous with Ag feeding modestly enhanced the degree of tolerance in the peripheral lymphoid tissues, as shown by increased suppression of proliferative responses after in vitro re-stimulation, IL-12 negatively regulates two of the main mechanisms of oral tolerance, TGF-beta production and clonal deletion via apoptosis. in addition, they suggest that the combination of oral Ag feeding and systemic anti-IL-12 administration may be of benefit in the treatment of autoimmune diseases[12].

Result show high concentration of IL-1 alpha in both oral and intramuscular vaccination however it was higher in intramuscular rout than in oral rout, A number of observations support the hypothesis that the production and release of L-1alpha were as effective as for the induction of Ag-specific serum IgG, secretory IgG and IgA, systemic delayed-type hypersensitivity, and lymphocyte proliferative[13].

Th2 cytokine IL-10 was higher in intramuscular vaccinated animal and combined with high antibody concentration. Th2 cytokines IL-10 was higher in intramuscular rout than in mucosal rout and to be significant help in antibody production [14].IL-10 was considered important in induction oral tolerance[14,15].

Briefly, factors which favor the Th1 type of response (IFN-γ, IL-12, and intact cholera toxin abrogate mucosal tolerance, while factors which favor Th2 (IL-4 and IL-10) or Th3 (TGF-β) response enhance the development and persistence of mucosal tolerance [16,11].

It has been proposes that the role of mucosal tolerance is to provide immunologic homostasis in the gastrointestinal tract [17].

Mild rate of IgM isotype switching to IgG were noted in both of the immunization routes [18].

Animal models for immune tolerance are of primary important in biomedical researches. They are either those of neonates or that of mature conventional and / or transgenic [19]. Like murine model for lung transplantation [20], and human CD3 trasngenic mice [21]. The present lapin model of oral mucosal tolerance, mucosal application of *S. typhi* bacterin induce low specific agglutination, specific IgM, Specific IgG as well as a mild rate of class switching from IgM to IgG. The interleukine 1 alpha, IL-10 but not IL-12 were proved to be of use in evaluation of immune tolerance state in this developed model.Such developed model may has potential bearing for use for therapeutic trends in hypersensitivity, autoimmunity as well as allograft sustainment [22].

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