An oral vaccine for type 1 diabetes based on live attenuated Salmonella

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A B S T R A C T

Type 1 diabetes (T1D) is a metabolic disease that is initiated by the autoimmune destruction of pancreatic insulin-producing beta cells that is accompanied by the development of antigen-specific antibodies and cytotoxic T lymphocytes (CTLs). Several studies have shown that vaccination with diabetic autoantigens provides some protection against this process. In this report we describe a new oral vaccine that utilizes live attenuated Salmonella for simultaneous delivery of autoantigens in conjunction with immunomodulatory cytokine genes to immune cells in the gut mucosa. Recent data showed that live attenuated Salmonella is a safe, simple and effective vector for expression of antigens and cytokines by antigen-presenting cells (APCs) of gut-associated lymphatic tissue (GALT). This novel strategy was tested by fusion of the diabetic autoantigen preproinsulin with Salmonella secretory effector protein (SseF) of pathogenicity island-2 (SPI2). In this way the autoantigen is only expressed inside the host immune cells and translocated to the host cell cytosol. In addition Salmonella was used to deliver the gene for the immunomodulatory cytokine transforming growth factor beta (TGFβ) for host cell expression. Oral co-vaccination of 8 week-old non-obese diabetic (NOD) mice with three weekly doses of both the autoantigen and cytokine significantly reduced the development of diabetes, improved the response to glucose challenge, preserved beta cell mass, and reduced the severity of insulitis compared with controls and autoantigen alone. Combination therapy also resulted in increased circulating levels of IL10 four weeks post-vaccination and IL2 for 12 weeks post-vaccination, but without effect on proinflammatory cytokines IL6, IL12(p70), IL17 and IFNγ. However, in non-responders there was a significant rise in IL12 compared with responders. Future studies will examine the mechanism of this vaccination strategy in more detail. In conclusion, Salmonella-based oral vaccines expressing autoantigens combined with immunomodulatory cytokines appears to be a promising therapy for prevention of T1D.

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1. Introduction

Central to the onset and progression of type 1 diabetes (T1D) is the loss of tolerance to specific self-antigens, resulting in immune-mediated destruction of insulin-producing cells. Therefore, a major theme of current diabetes research is restoration of balance within the immune system using treatments ranging from systemic immunosuppression to activation or inhibition of specific immune signaling pathways to infusion of specific regulatory cell populations [1,2]. One promising approach is vaccination with diabetic autoantigens, which can inhibit destructive islet-specific responses and induce regulatory responses [3,4]. Moreover, co-administration of certain cytokines synergizes with the antigen-specific effect [5,6]. However, the efficacy of vaccination with autoantigens is dependent on the many factors including the route of administration, the dose and duration of treatment, and the composition of the vaccine.

Many diabetic autoantigens have been identified [7], such as proinsulin [8], glutamic acid decarboxylase (GAD65 from the GAD2 gene) [9], islet-specific glucose-6-phosphatase [10], chromogranin A [11], and islet amyloid polypeptide [12]. The most studied of these are proinsulin and GAD65, both of which are very effective in reversing and preventing diabetes in non-obese diabetic (NOD) mouse model of T1D, though somewhat disappointing when translated to clinical trials [13,14]. A recent example was vaccination with GAD-Alum which induced regulatory T cells [15] and delayed the loss of glucose-stimulated C-peptide levels in patients treated soon after diagnosis, but did not prevent the disease [16,17]. While
disheartening, these results indicate that autoantigen-based therapies in humans can restore immune balance when administered in the right context and with an optimal regimen.

Oral delivery of antigens has long been recognized as an effective route for induction of tolerizing effects. The gut-associated lymphoid tissue (GALT) is faced daily with massive levels of antigens arising from diet and intestinal flora, and yet suppresses inappropriate escalation of inflammatory responses by a tendency toward tolerance [18,19]. This tendency has been exploited in the development of promising treatments for autoimmune diseases [18]. An emerging method of safe and effective delivery of oral antigen-specific vaccines uses live attenuated (non-pathogenic) Salmonella typhimurium [20]. Salmonella carrying an antigen-expression plasmid are naturally transferred from the gut to phagosomes of GALT antigen presenting cells (APCs) where they form Salmonella-containing vacuoles (SCV). The bacteria remain viable and multiply inside the SCV and deliver the recombinant antigen into the host cell cytoplasm, thus avoiding intestinal degradation of antigen [21–24]. Utilization of the Salmonella pathogenicity island-2 (SPI2)-encoded type III secretion system (T3SS) [25] for antigen delivery delays antigen expression until the bacteria are taken up by APCs, which increases safety and efficacy. The APCs process and present antigen to other immune cells in the gut and then migrate to other organs [26,27]. Such vaccines have been shown to be very effective in eliciting both CD8 and CD4 T cell-mediated immune responses in models of infectious diseases and cancer [28,29]. In fact, Salmonella is being used for the development of cancer vaccines with promising results, though maximum effectiveness requires addition of immunostimulatory agents to augment the cytotoxic effect [21,30].

Beyond antigen expression, Salmonella can also be used as a “DNA vaccine” system to deliver expression vectors that are directly expressed by the host cell [31]. Therefore, this Salmonella-based approach can accommodate various combinations of antigens and host-expressed modulators for optimization of treatment regimens. The flexibility of the system allows rapid development of new immunotherapies which provide robust and durable delivery of multiple antigens in a safe and inexpensive manner.

This study describes the development and testing of SPI2-T3SS of Salmonella for delivery of diabetic autoantigens. We chose preproinsulin as the autoantigen to test this system since it has already been demonstrated to be an effective antigen-specific treatment in the NOD mouse model of T1D, and this was combined with delivery by Salmonella of a plasmid for host expression of transforming growth factor-beta-1 (TGFβ). Oral vaccination with combined autoantigen/cytokine prevented development of autoimmune mediated diabetes in the majority of mice for more than 25 weeks post-vaccination.

2. Materials and methods

2.1. Preparation of Salmonella vaccines

This study used the double mutant strain of S. typhimurium MV728 (ΔhtrAΔapurD) as previously described [21,30]. Bacteria were cultured in Luria-Bertani (LB). If required for selection, ampicillin (100 μg/ml), kanamycin and/or carbenicillin (50 μg/ml) was added. Plasmids containing the open reading frames (ORFs) for mouse Ins2 (cat no.-MR226647), human GAD2 (cat no.-RC225984), mouse Tgb1 (cat no.-MR227339) and mouse IL10 (cat no.-MR2270340) were obtained from Origene (Rockville, MD). The Tgb1 and IL10 plasmids were used as is. The ORFs of Ins2 and GAD2 were sub-cloned into plasmid p2810 for expression under the control of the SPI2 promoter as a fusion protein with SseF which mediates translocation to the host cell cytoplasm [23]. Deep-Vent DNA polymerase (NEB) was used to amplify the mouse ins2 gene using oligonucleotides INS2-Hpal-For (5’-ATAGTTAACATGGCCGGACATT-3’) and Myc-SDK-Xbal-Rev (5’-TGCTCTAGATTAACACCTTGTCATGCCTGTG-3’) and human GAD2 gene using H-GAD65-Hpal-For (5’-ATAGTTAACATGGCCAATCCTG-3’) and Myc-SDK-Xbal-Rev from the Origene plasmids. The PCR fragments were sub-cloned into p2810 plasmid. The resulting plasmids pMH508 (preproinsulin) and pMH502 (GAD65) were confirmed by DNA sequencing and transformed into Salmonella MvP728 by electroporation (Bio-Rad MicroPulser).

2.2 In vitro infection

The murine RAW264.7 macrophage cell line was obtained from the American Type Culture Collection (ATCC no.-TIB-71), and maintained according to manufacturer instructions. Cultures were treated with Salmonella-expressing preproinsulin pMH508 (Sal-PPI) or GAD65 pMH502 (Sal-GAD65), or Salmonella-delivering TGFβ (Sal/TGFβ) or IL10 (Sal/IL10) at a multiplicity of infection of 10 for 25 min, washed, and cultured for 16–20 h for expression analysis [23]. The cells were immunostained as previously described [21].

2.3. Animal experiments

Seven week old female NOD/ShiLtJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained under specific pathogen-free conditions. Animals received high quality care consistent with Public Health Service Policy. The animal care facility at City of Hope has been fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The study was approved by the Institutional Animal Care and Use Committee (IACUC no. 11032).

Norglycemic NOD mice (8 weeks old) were grouped into 3 groups: Sal/TGFβ and Sal-PPI (n = 8), Sal-PPI alone (n = 7), and vehicle control (n = 5). Mice were vaccinated by oral gavage with 107 CFU/mouse of Sal/TGFβ and/or 105 CFU/mouse of Sal-PPI in a total volume of 200 μl of 5% sodium bicarbonate on days 0, 7, and 14.

Blood glucose was measured every 3–4 days with One Touch Ultra glucometer (LifeScan, Milpitas, CA). Mice were considered diabetic when two consecutive blood glucose values were at or above 200 mg/dl (11.1 mM). Intraperitoneal glucose tolerance tests (IPGTT) were done as previously described [32].

2.4. Insulitis score

At the end of experiment, pancreatic paraffin sections were stained with hematoxylin and eosin (H&E). Islets were observed under light microscopy at 20× or 40×, enumerated and graded in blinded fashion. Pancreatic sample were scored for islet infiltration as follows: 0 = no insulitis; 1 = peri-insulitis; 2 = mild insulitis with <50% islet area affected; 3 = invasive insulitis with >50% islet area affected; 4 = invasive insulitis with 100% islet area affected). Between 25 and 98 islets were scored from 4 mice of each group.

2.5. Immunostaining and cytometry

Pancreatic paraffin sections were immunostained for insulin and DNA. Parallel sections were quantitated by laser scanning cytometry using an iCys laser scanning cytometer (LSC) (CompuCyte, Westwood, MA) based on staining for nuclei (using a 405 laser) and insulin (using a 488 laser) essentially as described previously [33]. The whole sections were scanned for each slide. The
fraction of beta cells is shown as the percentage of insulin positive cells in the total number of cells.

2.6. Multiplex cytokine measurement

Circulating levels of IL-2, IL-6, IL-10, IL-12p(70), IL-17 and interferon-gamma (IFNγ) were measured in serum using a Multiplex Map kit (Millipore, Billerica, MA) and a Bio-Plex analyzer (Bio-Rad, Hercules, CA) in accordance with the protocol of the manufacturer.

2.7. Statistical analyses

Statistics were performed using GraphPad Prism 6 software and a value of P < 0.05 was considered significant.

3. Results

3.1 In vitro assessment of Salmonella-based expression

To evaluate the application of the Salmonella antigen-specific vaccine methodology to treatment of T1D, four Salmonella-based vector strains were made: two expressing autoantigens (preproinsulin (PPI) and GAD65) (Fig. 1A), and the other two mediating host expression of tolerogenic cytokines (TGFβ and interleukin10 (IL10); Fig. 1B). Salmonella transformed with the plasmids were used for in vitro infection of RAW264.7 macrophages followed by immunofluorescence staining (Fig. 2). As shown, all four strains of Salmonella were taken up by the macrophages in culture and are present in the cytoplasm (Fig. 2, green). In the case of the autoantigens the SPI2 promoter (PsseA) is activated after internalization of the bacteria by the macrophages causing expression of the fusion proteins. Infection with Sal-PPI (Salmonella expressing SseF-preproinsulin) or Sal-GAD65 (Salmonella expressing SseF-GAD65) results in appearance of the autoantigen (red) in the cytoplasm of the host cells which co-localizes with the bacteria (Fig. 2B). Conversely, Salmonella carrying plasmids for TGFβ (Sal/TGFβ) and IL10 (Sal/IL10) mediate host cell expression of cytokines (red) which co-localize with loci of intracellular bacterial replication (Fig. 2C). The results demonstrate that these constructs are competent to direct production of the respective proteins post-infection.

3.2. Prevention of diabetes in NOD mice treated with oral Salmonella-based vaccine

In vivo assessment of the vaccination methodology was tested using PPI as autoantigen in combination with TGFβ. Normoglycemic NOD mice (8 weeks) were treated orally for three consecutive weeks with Sal-PPI combined with or without Sal/TGFβ. Control mice were treated with vehicle alone. Fig 3A shows that combination therapy using Sal-PPI plus Sal/TGFβ prevented the development of T1D for more than 25 weeks in the majority of animals (75%), but only 20% of the control mice and no mice treated with Sal-PPI alone remained diabetes-free. In conjunction with this, blood glucose levels in control and antigen-alone vaccinated mice became highly unstable starting at day 75 post-vaccination, but remained stable throughout the study in mice treated with the combination therapy (Fig. 3B). The mice showed no signs of adverse effects of the vaccine and maintained normal weight gain throughout the study (Fig. 3C).

3.3. Intraperitoneal glucose tolerance tests (IPGTT)

IPGTT were performed on day 137 post-vaccination to further investigate the metabolic stability in each group of mice. The peak post-injection blood glucose level in both control and antigen-alone groups occurred at 30 min with means of 424 and 392 mg/dl, respectively. In addition the blood glucose levels failed to return to normal levels even after 120 min (Fig. 4A), indicating the development glucose intolerance in these mice. By contrast, the average peak blood glucose level in co-vaccinated mice was 294 mg/dl and returned to normal by 120 min (Fig. 4A). Area under the curve (AUC) quantification of the results show that co-vaccination prevented the severe glucose excursions found in the other groups (P < 0.05; Fig. 4B) and significantly improved the glucose disappearance rate [34,35](P = 0.01; Fig. 4C).

3.4. Co-vaccination preserved functional β cell mass and reduced severe insulitis

The level of lymphocyte infiltration was scored in paraffin sections from all treatment groups (Fig. 5A). Even prior to vaccination the mice exhibited insulitis (Fig. 5B). At 25 weeks post-treatment the unvaccinated control mice had severe insulitis consistent with the development of diabetes. Antigen alone (Sal-PPI) appeared to decrease insulitis overall but the change did not reach the level of significance and did not prevent diabetes. However, co-vaccination with Sal-PPI + Sal/TGFβ significantly reduced severe insulitis (Fig. 5B; two-way ANOVA, P = 0.038 at <50% and P = 0.0002 at 100%), and preserved higher percentage islet area than control or antigen alone (ANOVA, P = 0.048).

The percentage of insulin-positive cells was also quantified in the pancreas sections (Fig. 5C and D). The islets in both the control and antigen alone groups exhibited abnormal morphology and low level insulin staining (Fig. 5C). The control in particular had islets with few or no insulin positive cells. By contrast, the combination
treatment resulted in significantly higher percentages of insulin-positive cells compared to antigen alone and control (Fig. 5D; one-way ANOVA, P = 0.049 and P = 0.047, respectively).

3.5. Effect of combined therapy on cytokine profile

To evaluate the effect of combined vaccine therapy on the immune response, cytokine levels in the serum were measured before and after vaccination. The combined therapy, but not antigen alone, increased serum levels of IL10 and IL2 (Fig. 6A). IL10 rose to a significant level at 4 weeks post-vaccination and then returned to basal levels, but IL2 showed a significant increase for at least 12 weeks post-vaccination. In contrast, there was no significant effect on the levels of IL6, IL12(p70), IL17 or IFNγ. However, two of the co-vaccinated mice did not appear to respond to treatment, and comparison of responders (non-diabetic) and non-responders (diabetic) showed significant increases in serum levels of circulating IL12 in the diabetic animals, and elevation, though not significantly, of IL10, IL2, and IFNγ (Fig. 6B).

4. Discussion

The goal of the current study was to test whether a safe and effective method of preventative vaccination could be adapted to a tolerogenic purpose. This study demonstrated that oral co-vaccination with Salmonella-based preproinsulin and TGFβ reduced insulinitis, preserved insulin-positive cells, and prevented diabetes in NOD mice. The vaccine exploited two different modes by which Salmonella can deliver molecules to the host: First, autoantigen was expressed using the Salmonella SPI2 system which mediates translocation to the cytosol of host APCs for antigen processing. Second, Salmonella delivered a plasmid encoding the cytokine for expression by the host APCs.

Successful vaccine development requires the convergence of several key components, and we believe that for tolerance some of these are found in the unique properties of the gut-associated immune system. GALT dendritic cells have distinct qualities that distinguish them from central APCs [36] and which favor immunomodulatory responses including generation of extrathymic antigen-specific Tregs [2,27]. Several studies have shown that GALT APCs process and present antigen to other immune cells in the gut and then migrate to other organs such as mesenteric and pancreatic lymph nodes where they participate in peripheral immune responses [26,27]. The tolerogenic tendency of the GALT has been exploited in the development of promising oral treatments for autoimmune diseases [18] but a chief difficulty in this approach is that the antigen is exposed to degradation in the stomach and intestines which is difficult to quantify and control. A great advantage of the current approach is that antigen expression is under the control of a SPI2 promoter which is only activated after
Fig. 3. Co-vaccination reduces incidence of T1D and prevents hyperglycemia. NOD mice were treated orally with Sal-PPI combined with Sal/TGFβ (n = 8, square), Sal-PPI alone (n = 7, triangle), or with vehicle (n = 5, circle). Boost vaccination was given on days 7 and 14. Blood glucose level was monitored every 3 to 4 days.  (A) Log-rank plot of the percentage of NOD mice that remained diabetes-free over the time course of the study. The differences between the group of mice vaccinated with combined therapy and other groups was significant (P<0.05) by the log-rank (Mantel–Cox) test. (B) The average of blood glucose level and (C) the average of body weights for each group of mice over the time of the experiment. Each time point represents the mean and ±SEM of 5–8 samples. Statistical analysis using two-way ANOVA shows the significance between combined therapy and control group (** P<0.005, *** P<0.0001, and **** P<0.0001), or antigen alone (P<0.05, ** P<0.005, and *** P<0.001).

Fig. 4. Co-vaccination prevents glucose intolerance. (A) Intraperitoneal glucose tolerance tests (IPGTT) were done as previously described [32], briefly IPGTT were performed at day 137 post-vaccination for mice vaccinated with combined therapy (n = 6, square), antigen alone (n = 4, triangle), and the control group (n = 3, circle). Mice were fasted overnight (16 h) with access to water. Blood glucose levels were measured before and at 5, 10, 20, 30, 60, or 120 min after intraperitoneal injection of 2.4 g glucose/kg body weight. (B) AUC glucose was calculated over 120 min using fasting blood glucose as the basal level. (C) Glucose disappearance rates were calculated by measuring K-value of blood glucose peak at 30 min to the end of the assay at 120 min (the slope of natural log (ln) of blood glucose versus minutes is the K-value) [34,35]. Each time point represents the mean and ±SEM of 3–6 samples. Asterisks indicate values that are significantly different from the group of mice vaccinated with combined therapy using statistical analysis of one-way ANOVA * P<0.05.
internalization of the bacterium and then the antigen is directly translocated into the cytoplasm of the host APC.

As noted earlier, this characteristic of Salmonella-based vaccines has been previously used for generation of protective immune responses [21,23,38]. In examining the results of these studies, it became apparent that oral delivery of heterologous antigen alone was inadequate to initiate an effective immune response especially in the case of tumor killing, and only with the addition of agents such as glucuronosylceramide [21] or STAT3 RNAi [30] to augment the response were the vaccines effective. Based on this observation, we postulated that this was due to induction of both protective and immunomodulatory mechanisms in the GALT, and that these stimulatory agents were required to shift the balance toward a cytotoxic response, and therefore the inclusion of immunosuppressive factors like TGFβ may alternatively shift the balance toward a tolerogenic response. Furthermore, live intracellular bacteria were expected to provide more durable and stable expression of autoantigens and cytokines, and that prolonged expression might favor antigen-specific tolerance.

Other oral bacteria-based approaches are currently being explored as vectors for antigen-specific immunotherapies for diabetes. Most notably is the elegant study by Takishii et al. [39] using Lactococcus lactis to simultaneously deliver proinsulin and IL10. By co-administration of anti-CD3 the authors were able to induce antigen-specific tolerance and reverse early onset diabetes. However, a distinct advantage of Salmonella for vaccine development is that the antigen is only expressed after the bacteria are inside the host APCs due to the SPI2 promoter [20]. Bypassing expression of antigen in the intestinal lumen avoids degradation and loss of antigen, and also unwanted immune responses. Furthermore, internalization of Salmonella by dendritic cells ensures persistent antigen expression without dietary or other influences, which in the case of Lactococcus limits its ability to generate an antigen-specific response [40]. Since it is internalized, Salmonella, unlike Lactococcus, can also carry and transfer mammalian expression vectors to be expressed by the host cells [31], providing an efficient method for local secretion of immunomodulators such as TGFβ in the current study.

Another benefit of using live bacteria for development of oral vaccines is the ease with which new vaccines can be generated and tested using standard molecular techniques. This allows investigation of several autoantigens alone and in combination, which may be beneficial for translation to human studies. In addition, it is just as easy to include other immunomodulators such as IL10. As for translation to the clinic, Salmonella-based vaccines have proven safe and effective, and FDA-approved oral attenuated Salmonella
A) Cytokine profiles

B) Cytokines levels for co-vaccinated group

Fig. 6. Effect of combined therapy on serum cytokine levels. (A) Serum was collected from groups of animals before (pre-vac) and after (post-vac) vaccination at the time indicated and the levels of IFNγ, IL2, IL12(p70), IL10, IL6 and IL17 were measured. Each time point represents the mean and ±SEM of 6 co-vaccinated mice (square) and 7 mice vaccinated with antigen alone (triangle). (B) Comparison the cytokine levels between co-vaccinated diabetic (black bar) and non-diabetic mice (white bar). Significant differences were determined by two-way ANOVA. * \(P < 0.05\) difference between pre- and post-vaccination. \# \(P < 0.05\) differences between combined therapy and antigen alone.

vaccine is currently being produced by Berna Biotech (Switzerland) and available in the United States (Vivotif, Crucell Vaccines, Miami Lakes, FL) [41].

A potential confounding factor is the development of an immune response against the vector (i.e. bacteria strain) used to deliver the heterologous antigen, which can suppress the response to booster treatments. This has been observed with S. typhi Ty21A (Vivotif) vaccine and was suggested to result from suppression of bacterial multiplication, prevention of adhesion to the mucosa epithelium, and inhibition of invasion of the mucosa [42,43]. Furthermore, pre-existing immunity, or pre-exposure to related strains, can also diminish the response to heterologous antigens delivered in the same or similar vectors [44]. However, the effect appears to depend on several factors [45] including the strength of the response to the primary vaccination [43], as well as the specific strain and the specific antigen [46], and in some cases prior exposure to the vector enhances the response to the heterologous antigen [47]. Nevertheless, an effective strategy to overcome this limitation is to prime using one strain and boost with another strain expressing the same antigen. Sevil Domènech et al. [48] have demonstrated that this approach was effective even using S. typhimurium (antigen O group B) followed by Salmonella dublin (antigen O group D), and vice versa. Still, all of these studies examined the immune response to protective vaccines, so the effect of pre-exposure in the context of a tolerogenic vaccine will have to be investigated.

Several studies have suggested a direct relationship between different cytokines and the induction of mucosal tolerance in NOD mouse and our results show significant increases in serum levels of IL2 and IL10 after combined vaccine therapy (Fig. 6). In mice who did not respond to therapy there was a significant increase in IL12(p70) and elevation of IL2 and IFNγ. However, we are not able to assign specific effects to these molecules since their activities depend on context. For example, IL2 is necessary for the expansion and differentiation of CTLs and many immunosuppressives act by blocking IL2 production or signaling, but IL2 also has a critical role in promoting CD4+ CD25+ Treg cell survival and function [49] and T1D is associated with defects in IL2 pathway [50]. IL10 alone has contradictory effects in NOD mice [39,51-54], and it may be context-specific. In summary, increased levels of IL2 and IL10 in response to combined therapy are consistent with a shift toward tolerance, but the specific mechanism will be the subject of future studies.

In conclusion, we have adapted the oral Salmonella SPI2-based vaccine to induce tolerance and shown that it is effective to preserve insulin-positive cells and prevent diabetes in the majority of NOD mice. This is the first demonstration of using Salmonella as a vector vaccine to induce tolerance. The mechanism of vaccine action and whether this is an antigen-specific effect is under current investigation. In the future, this approach will be extended to other autoantigens and immunomodulators, possibly to generation of autoantigen libraries, and therefore to more effective combination treatments or even other autoimmune diseases.

Conflict of interest statement

The authors report that there are no potential conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.02.070.

References


