Systemic Immune Responses Induced After Immunization with HSV-2 Antigens Serve as Surrogates for Responses in the Murine Genital Tract

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ABSTRACT

GEN-003 is a subunit vaccine currently in phase 2 clinical trials for immunotherapy of HSV-2-induced genital herpes. Here we describe several methods to assess both humoral and T cell responses in murine genital tracts that can further characterize GEN-003 immunogenicity and support future vaccine development.

Extending our assessment of humoral responses by quantifying serum IgG responses, we developed a miniaturized ELISA method that measures both IgG and secretory IgA antibodies in vaginal washes of immunized mice. After vaccination with GEN-003 and adjuvant GBA1, mucosal IgG antibodies specific for both gD and ICP4 antigens were induced at comparable levels, mirroring systemic IgG responses in these animals. Secretory IgA responses tended to be greater for gD than ICP4, although this difference was non-significant.

Similarly, antigen-specific IFNγ responses in cell preparations from spleens or genital tracts of mice immunized with GEN 003 or a novel antigen GB209 together with adjuvant GBA2 followed the same patterns when quantified by ELISpot. Flow cytometry revealed these genital tract responses were derived from $CD4^+$ T cells, as relative frequencies of $CD3^+/CD4^+/IFN\gamma^+$ cells after restimulation with overlapping peptides spanning the sequences of gD, ICP4 and GB209 matched ELISpot counts. In addition, most of these antigen-specific T cells were polyfunctional, expressing at least three out of the four markers measured (IFNγ, IL-2, TNFα, CD107). As for antibodies, local polyfunctional $CD4^+$ T cell responses were accordant with systemic responses.

While intracellular cytokine staining of genital tract lymphocytes is feasible, cell quantities limit its regular use during vaccine candidate screening. Nevertheless, it is a powerful tool to assess immunogenic profiles of selected candidates.

Our data show comparable results of antigen-specific humoral and cellular immune responses locally in genital tracts and systemically in sera and spleens. Thus, systemic immunity can be used as surrogate for mucosal responses during early vaccine development in mice.

INTRODUCTION

Genocea's subunit vaccine GEN-003 is currently in phase 2 clinical trials for the treatment of recurrent genital herpes caused by HSV-2. It is comprised of two antigens, ICP4.2 and gD2ΔTMR, and the saponin-based adjuvant Matrix-M2[1]. In a dose optimization trial, GEN-003 reduced genital lesion rates by up to 65 % after 12 months as compared to pre-dosing baseline levels[2].

While the systemic immune responses to GEN-003 have been well characterized in animal models and humans, the understanding of local immune response in the genital mucosa is less complete. GEN-003 is uniquely designed to not only boost vaccine antigen-specific antibody titers, but also to stimulate T cell responses[3], whose role in disease control is increasingly appreciated[4]. In this context, the analysis of immune responses in the genital tract will provide deeper insight into the mechanisms of action of GEN-003.

In addition, the characterization of mucosal immune responses may also facilitate the rational design of novel vaccine candidates with improved efficacy. These next generation vaccines may contain different or additional antigens as well as an adjuvant with an improved activity profile.

Here, we describe both humoral and T cell responses in the genital tract of mice immunized with several vaccine candidates and compare them to systemic responses to vaccination.

Immunization Schemes

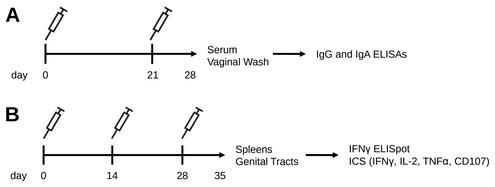

Figure 1: Study Designs. C57BL/6 mice were immunized subcutaneously on the indicated study days. One week after the last immunization, animals were sacrificed and the listed tissues were collected. (**A**) Antigen-specific IgG or IgA antibodies were quantified in sera and vaginal lavages using ELISA. (**B**) Interferon γ (IFNγ) responses were measured by ELISpot after restimulation of splenocytes or genital tract-derived lymphocytes with antigen-specific overlapping peptides. In addition, these cells were stained for the intracellular markers interleukin-2 (IL-2), tumor necrosis factor α (TNFα) and CD107a/b (CD107) and analyzed by flow cytometry with respect to their polyfunctionality (intracellular cytokine staining, ICS).

Vaginal and Systemic IgG Antibodies Are Induced in Parallel

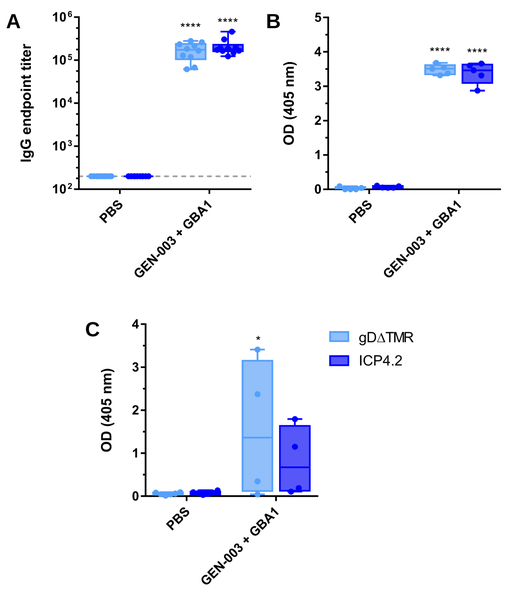

Figure 2: Antibody Responses to GEN-003. Animals were immunized with GEN-003 antigens together with GBA1 adjuvant or negative control, following the immunization scheme shown in Fig. 1A. Antibodies with specificity for either gD2ΔTMR or ICP4.2 antigens were measured separately. (**A**) Serum IgG were quantified by standard ELISA and endpoint titers were calculated using linear regression. The lower limit of quantification is indicated by a dashed line. (**B**) IgG and (**C**) IgA were detected in undiluted vaginal lavages using an analogous ELISA protocol. Raw optical densities (OD) are shown. Statistical analysis with Kruskal-Wallis test vs. the same antigen in the negative control group; * $p < 0.05$, **** $p < 0.0001$.

Vaginal IFNγ Responses Follow the Same Pattern as Systemic IFNγ Responses

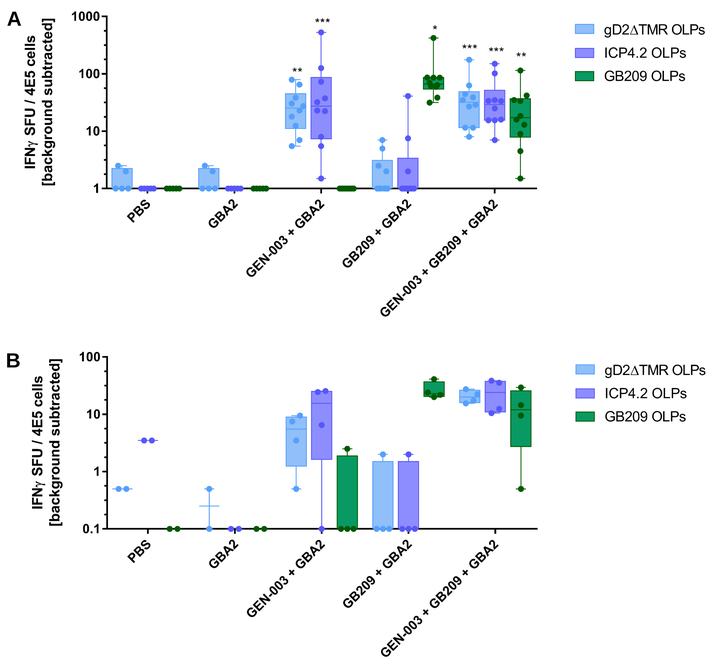

Figure 3: IFNγ Responses to GEN-003 and GB209, Quantified by ELISpot. Animals were immunized with GEN-003, GB209 or the combination of the two together with adjuvant GBA2. The immunization scheme shown in Fig. 1B was used. IFNγ responses specific for each of the antigens were quantified using ELISpot after restimulation with overlapping peptides (OLPs) covering the antigen sequences for 18h. Medium stimulation was used as a negative control. (**A**) Medium-subtracted IFNγ spot-forming units (SFU) are shown for splenocytes collected from individual mice. Statistical analysis with Kruskal-Wallis test vs. the same restimulation conditions in the GBA2 group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (**B**) Lymphocytes were isolated from genital tracts and pooled from 5 mice per sample. Medium-subtracted SFU for each replicate measurement are shown. Statistics are not calculated due to low number of biological replicates.

IFNγ-producing Lymphocytes in the Genital Tract Are Mainly $CD4^+$ T cells

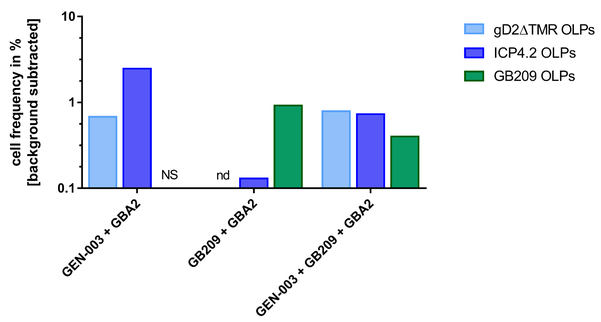

Figure 4: IFNγ Responses to GEN-003 and GB209 in Genital Tracts, Quantified by Flow Cytometry. Animals were immunized with GEN-003, GB209 or their combination with adjuvant GBA2, following the immunization scheme in Fig. 1B. Lymphocytes were isolated from genital tracts and pooled from all 10 animals per group. After 6h restimulation with OLPs for all three antigens, IFNγ-positive T cells were quantified pre-gated on live $CD3^+$, $CD4^+$, $CD14^-$, $CD117^-$, $GR1^-$, $CD19^-$, $CD45R/B220^-$, $NK1.1^-$ lymphocytes. $CD3^+$ $CD4^+$ $IFN\gamma^+$ cell frequencies in percent are shown after subtraction of frequencies achieved with medium restimulation. Statistics are not calculated due to low number of biological replicates. NS, no sample; nd, non-detectable.

There were no differences in background-subtracted frequencies of $CD3^+$ $CD8^+$ $IFN\gamma^+$ T cells after restimulation with the three antigens (not shown), indicating that $CD4^+$ T cells are the main producers of IFNγ after immunization.

Antigen-Specific $CD4^+$ T Cells Are Polyfunctional

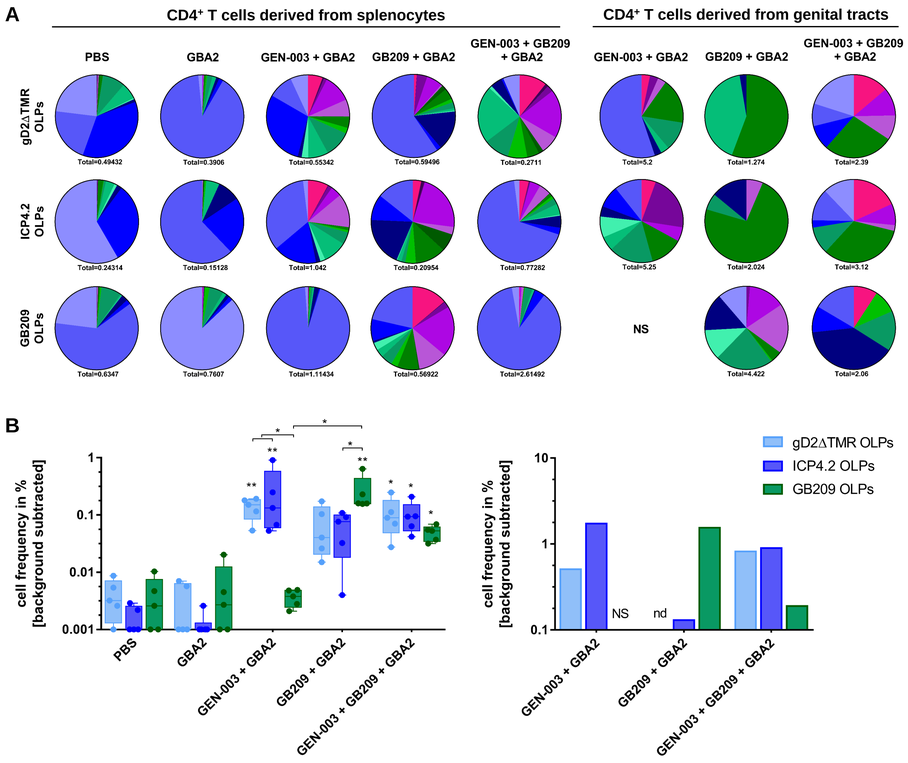

Figure 5: $CD4^+$ T Cell Polyfunctionality, Quantified by ICS. Cell were isolated as described in Fig. 3 and 4, restimulated with antigen-specific OLPs and medium for 6h and stained for both extra- and intracellular markers. Results for systemic T cells are shown on the left and for genital tract-derived cells on the right. $CD4^+$ T cells were identified as live $CD3^+$, $CD4^+$, $CD14^-$, $CD117^-$, $GR1^-$, $CD19^-$, $CD45R/B220^-$, $NK1.1^-$ lymphocytes. Antigen-specific expression of IFNγ, IL-2, TNFα, and CD107 was quantified as background-subtracted frequencies in percent. NS, no sample (**A**) Relative frequencies of all combinations of the 4 activation markers as shown. Quadruple-positive $CD4^+$ T cells, magenta; triple-positives, shades of purple, double-positives, shade of green, single-positives, shades of blue. Absolute frequencies are listed below each pie. (**B**) Absolute frequencies of $CD4^+$ T cells expressing 3 or more activation markers are shown. Statistical analysis with Kruskal-Wallis test vs. the same antigen in the GBA2 group unless otherwise indicated; * $p < 0.05$, ** $p < 0.01$. nd, non-detectable.

SUMMARY

After subcutaneous immunization, mucosal and systemic immune responses follow the same patterns, as shown here for several antigen/adjuvant combinations.

- Both serum IgG antibodies, implicated in protection from HSV-2 infection synergistically with T cells[5], and mucosal IgG, the predominant immunoglobulin in cervicovaginal mucus[6], were significantly boosted after immunization.
- Furthermore, antigen-specific T cells isolated from splenocytes and genital tract lymphocyte preparations show comparable activation profiles.

During development of novel HSV-2 vaccine candidates, immune responses to many antigen/adjuvant combinations will need to be characterized. Due to limited cell numbers isolated from genital tract tissues and the resulting need for sample pooling, regular assessment of T cell polyfunctionality in genital tissues is challenging.

Since local and systemic immune response correlate well, this difficulty can be overcome during initial screening of vaccine candidates by using systemic antibody and T cell responses as surrogates for those in the genital tract. For the most promising candidates, full analysis of local immune responses is still an important part for vaccine selection.

Future animal studies will aim at further characterizing the antigen-specific genital tract T cells induced after immunization with respect to their tissue residency, homing and protective capabilities.

References

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[2] Fife et al., ASM Microbe 2016
[3] Long et al., Virology, 2014
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[5] Morrison et al., J Virol, 2001
[6] Wang et al., Mucosal Immunol, 2014