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HIV-1 Env vaccine comprised of electroporated DNA and protein co-administered with Talabostat

Anthony D. Cristillo [a], Lindsey Galmin [a], Susana Restrepo [a], Lauren Hudacik [a], John Suschak [a], Brad Lewis [a], Ruxandra Draghia-Akli [b], Nazneen Aziz [c], Deborah Weiss [a], Phillip Markham [a], Ranajit Pal [a,*]

[a] Advanced BioScience Laboratories, Inc., 5510 Nicholson Lane, Kensington, MD 20895, USA
[b] VGX Pharmaceuticals, Inc., Immune Therapeutics Division, 2700 Research Forest Drive, The Woodlands, TX 77381, USA
[c] Point Therapeutics, 155 Federal Street, Boston, MA 02110, USA

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ABSTRACT

Selection of potent yet low reactogenic adjuvants for protein immunization is important for HIV-1 vaccine development. Immunogenicity of electroporated DNA (HIV *env*) and recombinant gp120, administered with either QS-21 or the orally administered immunomodulator, Talabostat, was evaluated in BALB/c mice. Electroporation of low dose DNA elicited Th1 cytokines and anti-envelope antibodies. Immunization with gp120 protein alone with or without Talabostat elicited lower Th1 and Th2 cytokine levels but comparable anti-gp120 antibodies to QS-21-formulated protein. Boosting of DNA-primed mice with gp120/Talabostat induced similar anti-gp120 antibody titers and slightly higher levels of Th1 and Th2 cytokines relative to QS-21-formulated protein. Induction of $CD8^+$ and $CD4^+$ T cells and functional CTL activity was noted. These results highlight the potential use of orally administered Talabostat for efficient protein boosting of antibody and T-cell responses primed by DNA.

Significant progress has been made in evaluating the immunogenicity of HIV-1 Env proteins using a DNA prime/protein boost vaccine regimen [1–7]. The degree of priming elicited by DNA immunization and the adjuvant co-administered with protein can greatly influence the level and quality of immune responses induced by these vaccines. Preclinical and clinical studies have shown that needle injection of DNA typically elicits weak antibody and T-cell responses which are enhanced by subsequent protein boost [3,4,6,8,9] or by different DNA delivery methods including gene gun, biojector, topical delivery, and electroporation [1,2,10–12].

To-date, numerous adjuvants have been tested in combination with HIV-1 vaccines including Alum [13], MF59 [14], lipid A [15], *Escherichia coli* heat-labile enterotoxin (LT) [16], cholera toxin (CT) [17], CpG deoxynucleotides [18], nanoparticles [19], and QS-21 [1–4,20,21]. While many adjuvants exhibit boosting properties when used in conjunction with subunit proteins, the degree of reactogenicity and tolerability in humans is a concern. In this communication, the boosting properties of Talabostat, an orally administered immunomodulator and aminoboronic dipeptide (L-valinyl-L-boroproline), was evaluated when co-administered with HIV-1 Env protein. Shown to be both safe and well tolerated in humans [22], Talabostat inhibits dipeptidyl peptidases and stimulates strong host immune responses, characterized by IFNγ, IL-1β, and IL-6 production, promoting innate, and adaptive immunity [23,24].

Materials and methods

Antigens/adjuvants. A CMV promoter-driven plasmid encoding codon optimized HIV-1 *env* (Ba-L) was used [3,25]. Recombinant gp120 (clade B) was expressed in stably transfected CHO cells and purified as described [3,4]. Protein was formulated with QS-21 adjuvant (Antigenics Inc., Woburn, MA) or administered with Talabostat (Point Therapeutics, Boston, MA).

Peptides. HIV-1 Env (Ba-L) peptide was synthesized (Infinity Biotech Research and Resource Inc, Aston, PA) as 79 peptides (15-mers) with 11 amino acid overlapping residues comprising the gp120 Env protein sequence. Peptides were resuspended in one peptide pool and cells were stimulated as indicated using a final peptide concentration of 1 μg/ml.

Electroporation of DNA. Mice were sedated, hair was removed from target areas and the plasmid solution was administered intramuscularly into the biceps femoris muscle using 3/10 cc syringe with 29 gauge needle. Following administration of plasmid, the CELLECTRA™ Adaptive Constant Electroporation Device (VGX Pharmaceuticals, Inc., The Woodlands, TX) was used for the electroporation of the vaccine. After a 4-s interval, electroporation was initiated with 0.1A constant current and two pulses of 52 ms/pulse at 1-s intervals.

Immunization protocols. BALB/c female mice (5–7 weeks old) were immunized by electroporation with either 20 μg, 10 μg or 5 μg of plasmid DNA at 0, 2, and 4 weeks. In a protein-only gp120/Talabostat optimization study, mice were immunized intramuscularly at weeks 0, 2, and 4 with gp120 (25 μg) and Talabostat (10 μg, 20 μg or 40 μg) was administered by oral gavaging (day0, day−1, day+1 relative to protein immunization). Alternatively, mice received gp120 (25 μg) protein formulated in QS-21 (20 μg). In the prime/boost study, mice were immu-

* Corresponding author. Fax: +1 301 468 9466.
E-mail address: ranajit.pal@ablinc.com (R. Pal).

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nized with electroporated DNA (20 μg) at weeks 0, 2, and 4 and boosted with gp120 protein, with QS-21 or Talabostat, at weeks 12 and 18. Mice ($n = 5$ per group) were sacrificed at 2 weeks post-final immunization and splenocytes and serum collected for immunological assays.

Binding antibody assay. Serum samples were tested for Env gp120- and Gag-specific antibodies using an enzyme-linked immunoabsorbent assay (ELISA) as described [26].

IFNγ ELISPOT. The IFNγ ELISPOT assay was performed with murine splenocytes according to the manufacturer's protocol (Ucytech, Utrecht, Netherlands) as described [3].

Cytometric bead array (CBA). CBA analysis was performed to quantitate secreted IFNγ, IL-2, TNFα, IL-4, and IL-5 from the supernatants of stimulated murine splenocytes according to the manufacturer's instructions (BD Biosciences, San Diego, CA). Splenocytes were stimulated for 24 h with either Env peptide pool (1 μg/ml) or with PMA (50 ng/ml) plus Ionomycin (1 μg/ml). Cytokine levels were quantitated using BD 5-bead Analysis Software. Th1 and Th2 cytokine responses were not detected in supernatants from naïve mice splenocytes stimulated with peptides (data not shown).

CTL activity. CTL activity was assessed using a single cell-based two color flow cytometric cytotoxicity assay, CytoToxilux® (OncoImmunin, Inc., Gaithersburg, MD) as previously described [2].

Intracellular cytokine staining (ICS). Splenocytes were stimulated for 5 h with Env peptide pool (1 μg/ml) or PMA (50 ng/ml) plus Ionomycin (1 μg/ml) in the presence of Golgi Plug (1 μg/ml; BD Biosciences). Following stimulation, cells were stained with anti-mouse αCD3-FITC, αCD4-PerCP and αCD8-APC monoclonal antibodies (BD Biosciences). A mixture of Th1 (TNFα–PE, IFNγ–PE, and IL-2–PE) or Th2 (IL-4–PE and IL-5–PE) cytokine antibodies (BD Biosciences) was used for cytokine detection followed by cytometry analysis.

Statistical analysis. Cellular data from murine studies evaluating three or more groups was analyzed by transforming (square root) data values in order to satisfy Bartlett's test for equal variances and then applying ANOVA/Tukey's post-test. Humoral data was transformed (log of data values) to satisfy Bartlett's test before applying ANOVA/Tukey's post-test. Cellular and humoral data obtained from studies evaluating two groups were analyzed using a two-tailed, unpaired *t*-test.

Results and discussions

Optimizing electroporated DNA-induced priming of T-cell and antibody responses

To optimize electroporated DNA administration, mice were immunized with varying DNA doses. IFNγ was quantified by ELISPOT following an 18 h *ex vivo* stimulation with gp120 Env peptide pool. Vaccine-specific IFNγ was found to be induced following three DNA immunizations and were statistically comparable ($p > 0.05$) in all groups (20 μg, 10 μg, and 5 μg) tested (Fig. 1A).

Additional Th1 (TNFα and IL-2) and Th2 (IL-5 and IL-4) cytokines were quantitated by CBA. TNFα, IFNγ, and IL-2 were all induced following DNA electroporation to comparable levels ($p > 0.05$) (Fig. 1B). In contrast, Th2 cytokines were not detected in any groups evaluated (data not shown).

In order to determine the relative contributions of $CD8^+$ and $CD4^+$ T cells in cellular responses observed, ICS was performed using a mixture of phycoerythrin-conjugated antibodies specific for Th1 (IFNγ, TNFα, and IL-2) cytokines. In splenocytes of mice electroporated with 20 μg of DNA, both $CD8^+$ and $CD4^+$ T cells producing Env-specific Th1 cytokines were detected (Fig. 1C). The level of anti-gp120 antibodies elicited following three electroporations was found to be comparable ($p > 0.05$) in all groups tested (Fig. 1D).

Overall, these data highlight the utility of DNA electroporation in priming both antibody and T-cell responses with low doses of DNA. These results are consistent with previous reports of studies conducted in rabbits and nonhuman primates [1,12]. Given that DNA can persist at the site of injection for prolonged periods [27], an immunization strategy in which both antibody and T-cell responses can be primed with relatively low doses of DNA may minimize reactogenicity concerns in humans.

Optimizing recombinant protein induced antibody and T-cell responses

Several reports describing possible reactogenicity and toxicity associated with the use of some adjuvants [28–34] prompted this study of Talabostat, shown to have immunomodulatory effects without reactogenicity or toxicity [23], in a DNA prime/protein boost HIV-1 vaccine setting. Earlier studies in this laboratory demonstrated that QS-21-formulated proteins can boost DNA-primed antibody and to a lesser extent T-cell responses [2–4]. A study was initiated to compare immune responses following protein immunization of mice with either Talabostat or QS-21. Modest IFNγ levels, measured by ELISPOT, were noted in mice receiving protein/Talabostat (Fig. 2A). Such responses were significantly less than those observed in the protein/QS-21-immunized mice ($p < 0.001$) and were not influenced by increasing Talabostat dose. Similar findings were noted when IFNγ was quantitated by CBA analysis (Fig. 2B). TNFα production, which was more notable than IFNγ, was found to be statistically similar ($p > 0.05$) in both protein/Talabostat and protein/QS-21 groups (Fig. 2B). Similarly, levels of IL-2 (Fig. 2B), IL-5, and IL-4 (Fig. 2C) were found to be comparable in all Talabostat- and QS-21-immunized mice. There was also no statistical difference in titers of anti-gp120 antibodies in mice receiving either increasing doses of Talabostat or in mice that received Talabostat versus QS-21 ($p > 0.05$) (Fig. 2D).

Induction of T-cell and antibody responses using a prime/boost strategy with Talabostat

Talabostat was then evaluated using an electroporated DNA prime/protein boost immunization strategy. Two weeks post-protein immunization, IFNγ levels were found to be greater in the splenocytes of mice that received Talabostat compared to QS-21 ($p < 0.001$) (Fig. 3A). As shown in Fig. 3B and C, a similar trend was noted for additional Th1 (TNFα, IFNγ, and IL-2) and Th2 (IL-5 and IL-4) cytokines, measured by CBA, yet statistical significance was only noted for IL-2 and IL-4. Induction of Env-specific Th1 (Fig. 3D) and Th2 (Fig. 3E) cytokines in both $CD8^+$ and $CD4^+$ T cells was noted in both adjuvant groups. In the Talabostat group, cytokine levels were modest in $CD8^+$ T cells and more pronounced in $CD4^+$ T cells. Furthermore, the percentage of peptide-specific $CD4^+Th1^+$ cells was found to be greater in the Talabostat-immunized mice compared to the QS-21 treated mice ($p < 0.05$). Given the importance of $CD4^+$ T-cell function in the maintenance of memory $CD8^+$ T cells [35], such induction of $CD4^+$ T-cell help by this vaccination strategy could ultimately give rise to long-term immune responses in immunized hosts.

Functional CTL activity, as measured by the percentage of caspase-positive target cells, was evaluated in peptide-expanded splenocytes (effectors). Unlike cytokine production, CTL was found to be greater ($p < 0.01$) in mice immunized with QS-21 as compared to Talabostat (Fig. 3F).

Antibody responses elicited following immunization with either Talabostat or QS-21 were assessed at 2 weeks post-final immunization and both adjuvant groups were found to yield comparable levels (Fig. 3G).

Overall, our findings suggest that Talabostat is comparable to QS-21 in augmenting cellular and antibody responses in an electroporated DNA prime/protein boost vaccine strategy. Consistent with induction of Th1 responses by Talabostat previously reported [23], this study demonstrated that gp120 protein immunization accompanied by orally administered Talabostat elicits responses characterized by induction of IFNγ, TNFα, and IL-2. This induction was less pronounced when mice were immunized with gp120/Talabostat alone as compared to when gp120/Talabostat was administered to DNA-primed mice. Furthermore, gp120 protein immunization

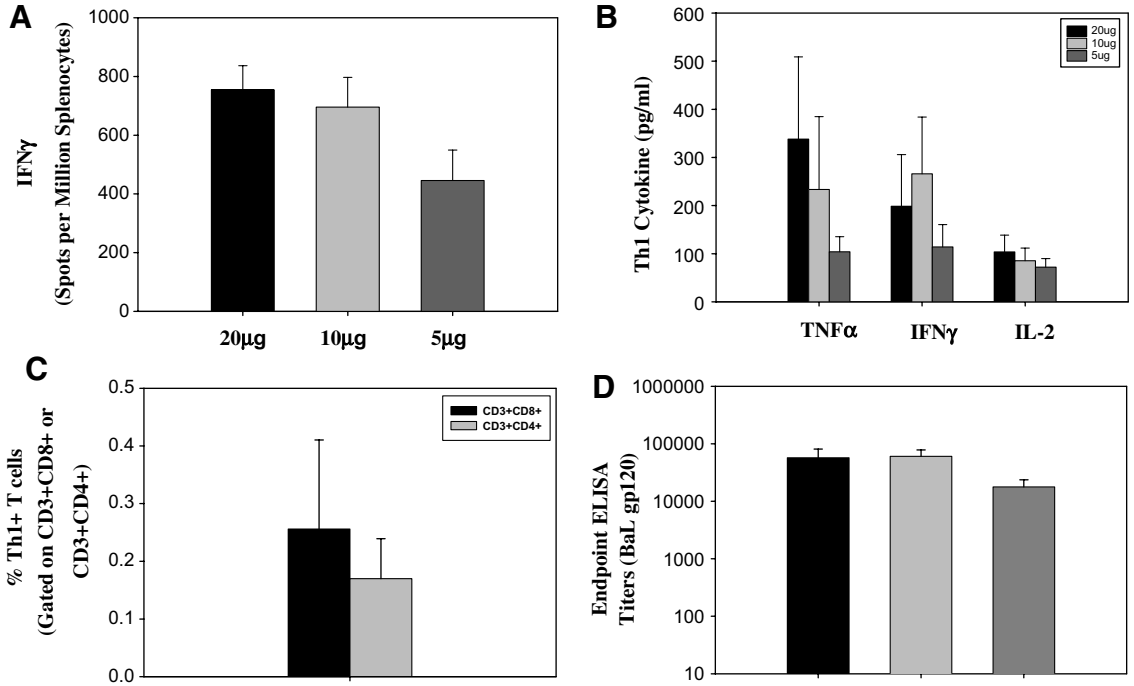

Fig. 1. Env-specific cellular and antibody responses in BALB/c mice immunized with electroporated DNA. Mice were immunized with 20 μg (black bars), 10 μg (light gray bars) or 5 μg (dark gray bars) of electroporated DNA on weeks 0, 2, and 4 and splenocytes collected on week 6 (Materials and methods). Cellular responses including IFNγ production (A), secreted Th1 (TNFα, IFNγ, and IL-2) cytokines (B), and intracellular Th1 cytokine production in $CD8^+$ and $CD4^+$ T cells were assayed from splenocytes of immunized mice following *ex vivo* Env peptide stimulation. Anti-gp120 antibody titers were also measured in the serum of immunized mice 2 weeks post-immunization (D). The average values ± standard error for each group ($n = 5$) are shown.

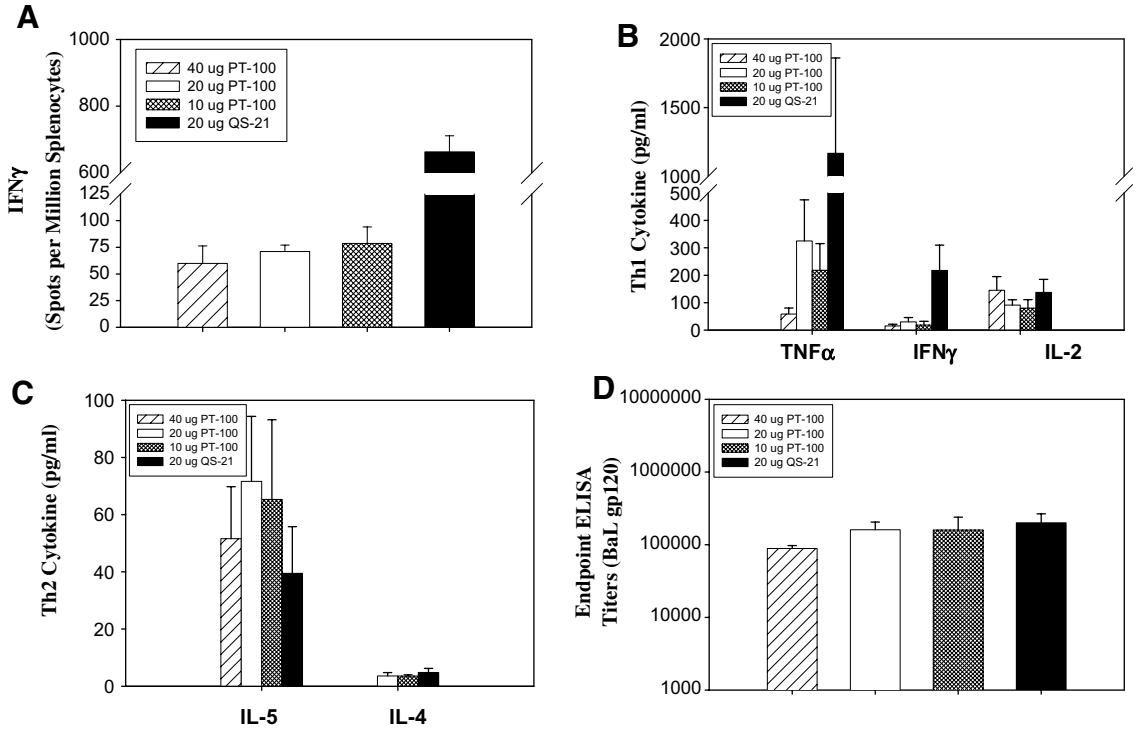

Fig. 2. T-cell and antibody responses elicited by Env protein immunization with Talabostat or QS-21. Mice immunized with gp120 protein at 0, 2, and 4 weeks were administered Talabostat (white bars, filled, and unfilled) by oral gavaging on the day prior to protein immunization, on the day of protein immunization and one day following protein immunization (Materials and methods). Alternatively, mice received recombinant gp120 protein formulated with QS-21 (black bars) adjuvant. Splenocytes and sera were collected at week 6 for immunological analysis. T-cell responses including IFNγ (A), secreted Th1 (TNFα, IFNγ, and IL-2) cytokines (B) and secreted Th2 (IL-5 and IL-4) cytokines (C) were assayed following *ex vivo* Env peptide stimulation. Anti-gp120 antibody titers were measured in the serum of immunized mice 2 weeks post-immunization (D). The average values ± standard error for each group ($n = 5$) are shown.

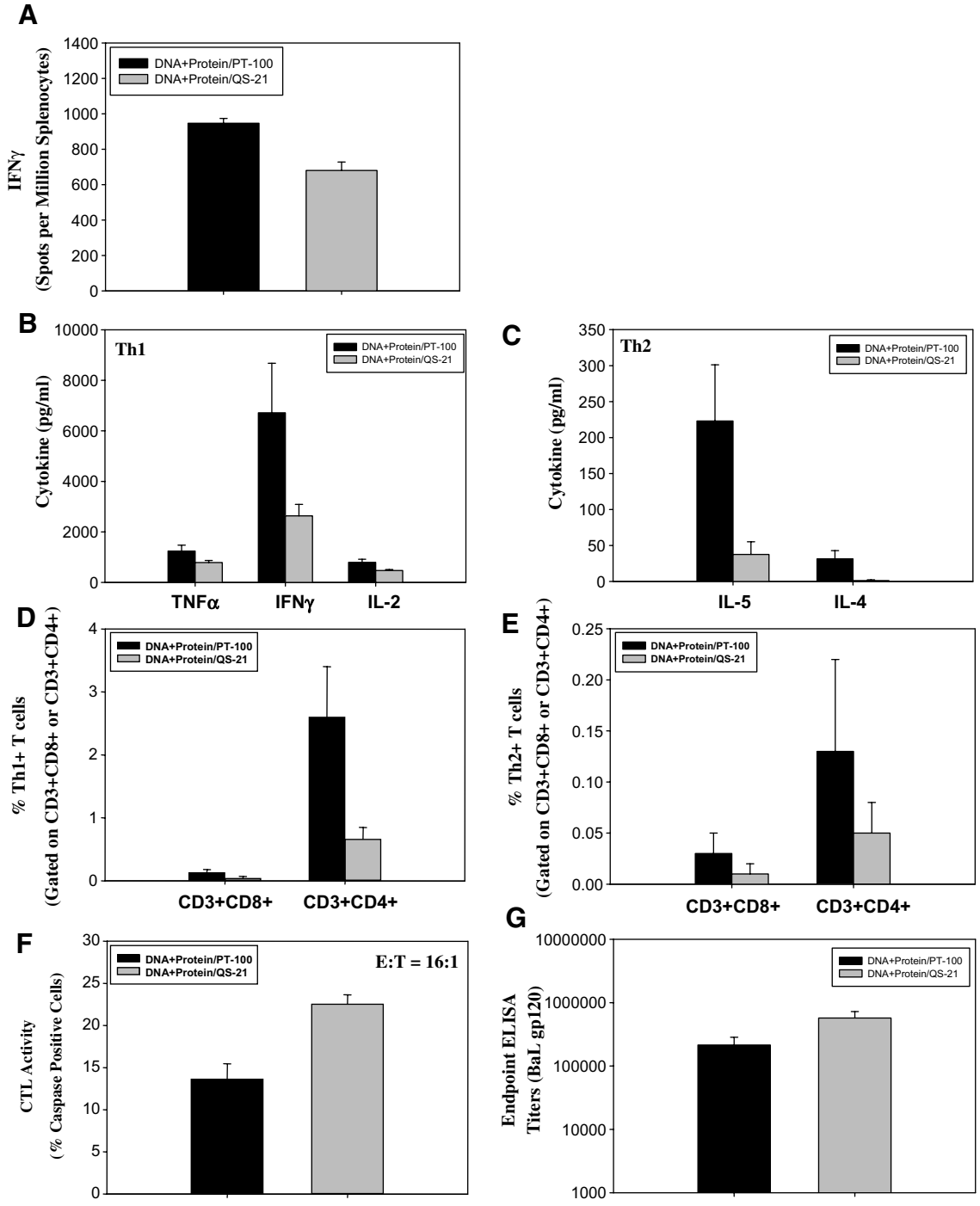

Fig. 3. Cellular and humoral responses elicited following electroporated DNA prime/protein boost immunization using Talabostat or QS-21 adjuvant. Mice were immunized at weeks 0, 2, and 4 with electroporated DNA (20 μg) and were boosted on weeks 12 and 18 with gp120 protein either formulated with QS-21 (gray bars) or co-administered with Talabostat (black bars) as outlined (Materials and methods). Splenocytes and sera were collected on week 20 for immunological testing. T-cell responses including IFNγ (A), secreted Th1 (B) and Th2 (C) cytokines, intracellular levels of Th1 (D) and Th2 (E) cytokines in $CD8^+$ and $CD4^+$ T cells, and CTL activity (F) were assayed following *ex vivo* Env peptide stimulation. Anti-gp120 antibody titers were also measured in the serum of immunized mice 2 weeks post-immunization (G). The average values ± standard error for each group ($n = 5$) are shown.

with Talabostat either alone (Fig. 2) or in combination with DNA priming (Fig. 3) also demonstrated induction of Th2 cytokines. Immune responses elicited by this approach were comparable or slightly greater than those noted following protein/QS-21 boost immunization and included a balanced Th1 and Th2 cytokine profile, CTL activity and CD4 T-cell help. These results highlight a promising vaccine approach that merits further investigation in preclinical studies with nonhuman primates.

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