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DNA, but not protein vaccine based on mutated BORIS antigen significantly inhibits tumor growth and prolongs the survival of mice

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Abstract

The ideal immunological target for cancer vaccine development would meet the criteria of tumor specificity, immunogenicity and vital dependency of the tumor on the functional activities of the antigenic target so as to avoid antigenic loss by mutation. Given that at face value the brother of regulator of imprinted sites (BORIS) transcription factor meets these criteria, we have developed a mutant variant of this molecule (mBORIS) that lacks tumorigenic ability, while retaining immunogenic epitopes that elicits responses against histologically irrelevant tumor cells. Here we compared vaccine strategies employing as an immunogen either mBORIS recombinant protein formulated in a strong Th1-type adjuvant, QuilA or DNA encoding this immunogen along with plasmids expressing interleukin (IL)12/IL18 molecular adjuvants. In both groups of vaccinated mice induction of tumor-specific immunity (antibody response, T-cell proliferation, cytokine production, T-cell cytotoxicity) as well as ability to inhibit growth of the aggressive breast cancer cell line and to prolong survival of vaccinated animals have been tested. We determined that DNA, but not recombinant protein vaccine, induced potent Th1-like T-cell recall responses that significantly inhibited tumor growth and prolongs the survival of vaccinated mice. These studies demonstrate that DNA immunization is superior to recombinant protein strategy and provide a clear guidance for clinical development of a cancer vaccine targeting what appears to be a universal tumor antigen.

Keywords

BORIS; 4T1 model; cancer immunotherapy; CT antigens

Immunotherapy of cancer offers the hope for a natural nontoxic alternative to current conventional approaches that are characterized by high morbidity, inefficiency against metastasis and development of drug resistance. One powerful strategy to overcome this hurdle is the combination of adoptive cell transfer, vaccination and chemokine/cytokine treatment.[1] Other strategies include enhancing of antitumor immunity by inhibition of immunosuppressive

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mechanisms,[2] use of different adjuvants,[3–5] novel carbohydrates mimetic peptides[6,7] or antigens involved in angiogenesis.[8,9]

A novel and very promising family of targets for antitumor immunotherapy, cancer testis (CT) antigens have been recently described and their expression has been observed in almost imaginable tumor types regardless of histology, with absent of expression in nonmalignant tissue with the exception of germ cells.[10–12]

A member of CT antigens family, so-called brother of regulator of imprinted sites (BORIS) is an epigenetically acting, tumor-promoting, transcription factor expressed in different types of human and mouse cancer cells has been recently described.[13–18] BORIS protein is absent in nonmalignant male tissues with the exception of testis, and is totally absent in females. The BORIS gene was first described as a DNA-binding protein that shares 11 zinc finger (ZF) domains with CCTC-binding factor (CTCF), but differs from this molecule at the N and C termini.[14] Other molecular studies suggest that aberrant expression of BORIS may serve as a previously unknown 'common denominator', which connects the molecular mechanisms involved in the initiation of epigenetic deregulation with those involved in triggering chromosomal instability and thus the 'mutator phenotype', the *sine qua non* of carcinogenesis. Derepression of BORIS gene expression as a result of oncogenesis is associated with expression of numerous CT genes, including *MAGEA2*, *MAGEB1*, *MAGEB4*, *GAGE-3–8*, *RAGE2*, *Oct3/4*, *LAGE1* and *NY-ESO-1*.[16] Importantly, another group confirmed that BORIS contributes to derepression of CT gene, *NY-ESO-1* in lung cancer cells and suggested that BORIS competes with CTCF for binding to the promoter of this gene.[17] Collectively these observations demonstrate that expression of *BORIS* in normal cells may result in demethylation and derepression of other CT genes[16,17] and strongly support previous data demonstrating that *BORIS* is abnormally activated in many different cancer cells.[13,14] Given that BORIS appears to be upstream of numerous molecular changes associated with oncogenesis, the development of an immunological strategy targeting BORIS is an attractive concept. In contrast to other tumor antigens, whose expression is not essential for tumor function, the immunologically mediated killing of BORIS-expressing cells may place the tumor in the proverbial bottleneck, with mutation causing lack of tumor function, and nonmutation causing immune-mediated death.

We have previously developed an immunogen comprising of a BORIS molecule lacking the DNA-binding ZF domain (mBORIS) so as to alleviate concerns of oncogenesis associated with immunization with wild-type BORIS.[15,18] DNA-based mBORIS (pmBORIS) vaccine elicited significantly stronger Th1-type of immune responses than recombinant mBORIS protein. In this study we further characterize immune responses induced by pmBORIS and mBORIS immunizations and for the first time identify and compare the potency of DNA- and recombinant protein-based strategies in a mouse model of mammary adenocarcinoma. More specifically we aim to evaluate ability of the optimized vaccine to inhibit *in vivo* growth of aggressive mammary carcinoma cells (4T1) and to prolong the survival of vaccinated mice.

Plasmid DNA vaccination was performed using the hEF1-HTLV promoter driving mBORIS placed in the pORF backbone (pmBORIS, as seen on Figure 1a). Additionally we optimize our DNA immunogen using well-characterized molecular adjuvants, interleukin (IL)12 and IL18 (Figure 1a). Plasmids encoding IL12 and IL18 were mixed with pmBORIS and injected to another groups of mice. DNA administration was performed by ballistic delivery using the Helios gene gun as we described earlier.[15,18] To compare the potency of this immunization strategy with recombinant protein vaccine we formulated the purified *Escherichia coli*-expressed mBORIS into the strong Th1-type adjuvant, Quil A (the protocol for immunization is depicted in Figure 1b). Thus, six groups of mice (n = 6 per group) were immunized with pmBORIS (9 μg per 6 μg antigen/vector per mouse); pmBORIS plus pIL12/IL18 (9 μg/3 μg/

3 μg per mouse); recombinant mBORIS protein (100 μg per mouse) formulated in Quil A (Sigma, St Louis, MA, USA); pIL12/IL18 (9 μg control vector plus 3 μg of each molecular adjuvants per mouse); QuilA alone (control) or phosphate-buffered saline (PBS) alone (naive).

Immunization with mBORIS protein in QuilA adjuvant induced potent antibody responses against mBORIS, strong T cell-proliferative recall response, and high IL-4 and low interferon (IFN)-γ production. Notably, BORIS-specific cytotoxic T lymphocyte (CTL) was not detected in splenocytes isolated from mice immunized with mBORIS protein (Table 1). In contrast, immunization with pmBORIS alone generated weak antibody responses, but still strong BORIS-specific T-cell proliferation, and a Th1-like cytokine profile characterized by high IFN-γ and low IL-4. Addition of molecular adjuvants (IL-12 and IL-18) resulted in similar characteristics of response as pmBORIS alone, with the exception that cytolytic activity was significantly higher (Table 1). These results indicate that even formulation of recombinant mBORIS into the strong Th1 adjuvant still induce mostly Th2 type of immune responses without generation of tumor-specific anti-mBORIS CTL. It is likely that such polarization of immune responses is due Th2-prone nature of BALB/c mice[19,20] used in our study. Most importantly our data essentially confirm our previous published results[18] and allowed us to analyze and compare the prophylactic anticancer potency of DNA- and protein-based mBORIS vaccines.

To analyze growth of tumors and survival of mice we first vaccinated all groups of control and experimental mice ($n = 12$ per group) exactly as described above in our immunological studies. Ten days after the last immunization all animals were implanted with 7×10^3 nonmodified syngeneic 4T1 cells into the mammary fat pad (in 50 μl PBS) of each mouse, and tumor growth rate and survival of mice were analyzed as described[15] (Figure 1c). Vaccination with pmBORIS/pIL12/IL18 as well as pmBORIS alone resulted in significant protection of mice from 4T1 tumor, although pmBORIS/pIL12/IL18 immunogen was more potent (Figures 2 and 3). Tumor growth was significantly inhibited in both groups of mice immunized with pmBORIS/pIL12/IL18 ($P<0.001$) and pmBORIS alone ($P<0.05$) (Figure 2). Although all experimental animals generated mammary fat pad tumors, 50% of mice from group immunized with pmBORIS/pIL12/IL18 and 37.5% of mice immunized with pmBORIS were still alive by day 41, whereas all control mice succumbed to tumor at this time point (Figure 3). In contrary to DNA immunization, vaccination with recombinant mBORIS protein, which induced Th2 type of immune responses did not inhibit tumor growth or prolong the survival of mice compared with controls. Thus, even in this model generally accepted as closely resembling clinical breast cancer,[21–25] DNA vaccine was capable of significant inhibition of tumor growth and prolongation of survival of mice after implantation of the very aggressive, highly metastatic and poorly immunogenic 4T1 tumor.

These data demonstrate that immunization with pmBORIS along with IL-12 and IL-18 molecular adjuvants appears to be a good strategy for generation of protective Th1-like immunity, including CTL responses. Through utilizing various immunization methods, such as protein versus DNA, or molecular adjuvant versus nonadjuvant, we have demonstrated that the immune response to mBORIS may be tailored toward either high antibody or high CTL profiles. This immune plasticity may be leveraged for further optimization of specific immune responses desired. Since wild-type BORIS is expressed intracellularly, we believe clinical entry of BORIS-targeting therapeutics should seek to evoke a strong CTL response and low antibody response. Given that antibody-mediated immunity to tumor antigens may actually enhance tumor growth,[26] clinical development of such vaccines should focus on strategies to minimize antibody production.

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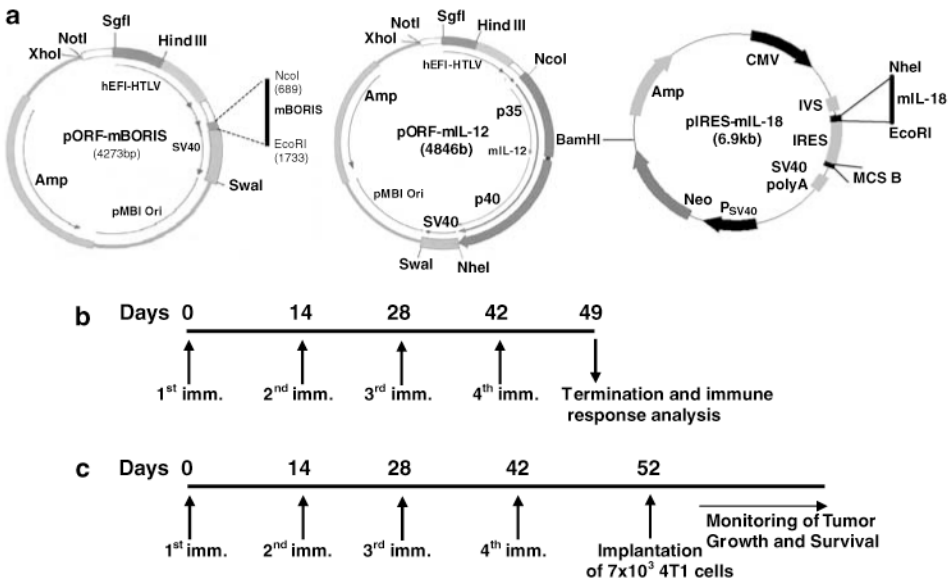

Figure 1.

(**a**) DNA plasmids encoding mBORIS, mouse interleukin (IL)-12 (mIL12) and mouse IL-18 (mIL18) used for immunization of mice. pORF-mBORIS was constructed as we described.[15] pORF-mIL-12 was purchased from Invivogen, San Diego, CA, USA. pIRES-mIL-18 was a gift from Dr Xiang (Scripps Research Institute, La Jolla, CA, USA); (**b**) experimental design of immunological studies; (**c**) experimental design of protective studies.

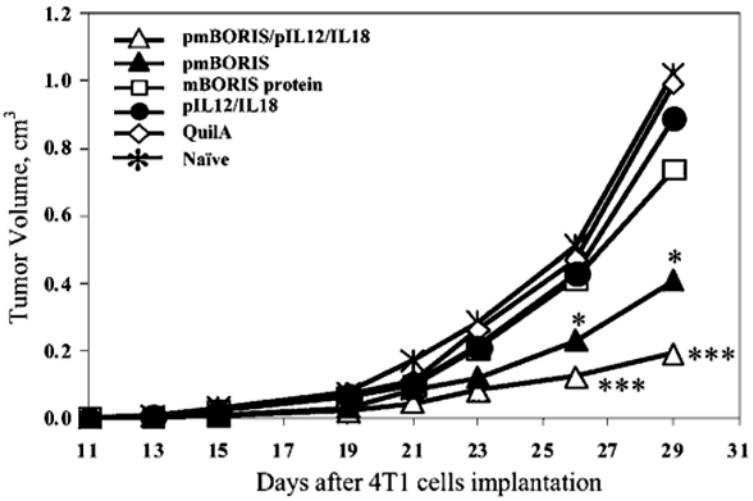

Figure 2.

Vaccination of BALB/c mice (n = 12) with pmBORIS and pmBORIS plus pIL12/pIL18 significantly inhibits tumor growth (*P<0.05 and ***P<0.001, respectively) on days 26 and 29 after implantation of 7×10^3 4T1 mammary carcinoma cells. Recombinant mBORIS protein did not inhibit the tumor growth. Tumor volumes were measured by two-dimensional measurement every 2–3 days, and calculated using the formula $L \times (W^2)/2$, where L represents the length and W the width of the tumor as described earlier.[15]

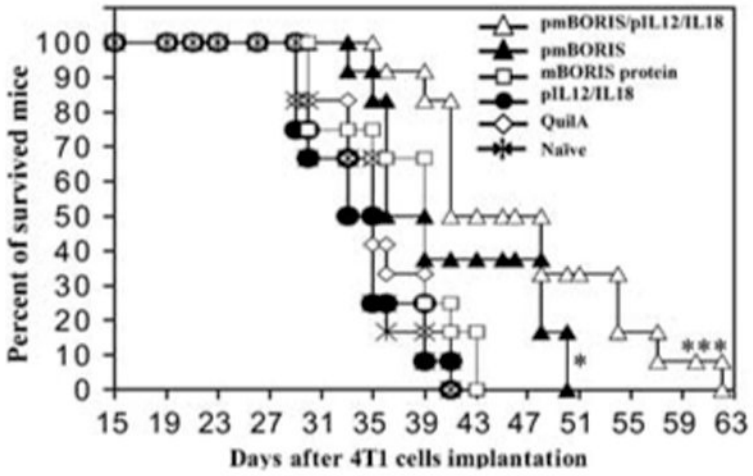

Figure 3.

Survival of mice (n = 12 per group) of unmodified mouse mammary carcinoma tumors in mice vaccinated with DNA or recombinant protein. Only immunization with pmBORIS or pmBORIS plus pIL12/pIL18, but not recombinant protein significantly prolongs the survival of mice (*P<0.05 and ***P<0.001, respectively) after implantation of 4T1 cells. On day 41, when mice in the control groups died from tumor growth, 50% of mice immunized with pmBORIS plus pIL12/pIL18 and 37.5% of mice immunized with pmBORIS were still alive.

Table 1

Comparison of immune responses generated by pmBORIS/pIL12/IL18 and pmBORIS versus recombinant mBORIS protein

Immunogen	Antibody response (dilution of sera, that gives OD_{450} ~0.2)	T-cell proliferation (stimulation index)	Cytokine-producing cells ELISPOT (no. of spots per 10^6 splenocytes)		Killing of 4T1 target cells at ratio 1:50
			IFN-γ	IL-4	
pmBORIS/pIL12/IL18	+/−	++	+++	+	+++
pmBORIS	+/−	++	+++	+	++
mBORIS protein	+++	++	++	+++	+/−

Abbreviations: IFN, interferon; IL, interleukin.

For antibody response: +/− titer from 1:100 to 1:400; +++ titer from 1:150 000 to 1:280 000; for T-cell proliferation: ++ stimulation index in range from 8.9 to 11.2; for ELISPOT assay: + from 70 to 100 spots per 10^6 splenocytes; ++ from 150 to 200 spots; +++ from 210 to 260 spots; for target cells killing: +/− from 0.5 to 1.5% of killing; ++ from 10 to 15% of killing; +++ from 25 to 30% of killing.