Table 1. Titration of Friend erythroleukaemia virus stocks

Virus dilution	Animals with erythroleukaemia [a]	
	FV-A	FV-P
10^{-1}	5/5	5/5
10^{-2}	5/5	5/5
10^{-3}	5/5	5/5
10^{-4}	5/5	5/5
10^{-5}	5/5	5/5
10^{-6}	2/5	5/5
10-7	0/5	0/5

[a]No. of mice with disease over No. of mice inoculated,

Table 2. Results of the second immunization experiment

Group	Animals with erythroleukaemia[a]	P
control	8/8	
pBSC	7/8	
pBSC/gag	2/10	P < 0.01
pBSC/env	2/10	P < 0.01
FLC 3cl8* (irradiated)	7/10	NS
FLC IFN-α cl11* (irradiated)	2/10	P < 0.01
FLC IFN-α cl11	0/7	P < 0.01

[a]see Table 1

First immunization experiment

In the first experiment plasmids pBSC/env, pBSC/gag or pBSC were administered either intramuscularly or intradermally. The mice were challenged with approximately 20 ELID50 of either A or P virus. As indicated in Fig. 3A and 3B, all control mice, whether untreated or injected with the control pBSC plasmid, developed the disease. A weak protection against virus-induced erythroleukaemia was observed both in mice immunized with pBSC/env and pBSC/gag. When taking into account mice challenged with both A and P viruses, *env* immunization protected 9 out of 19 mice, and *gag* immunization 2 out of 20 mice. Only the effect produced by pBSC/env was statistically significant ($P < 0.05$). While protection by pBSC/env against both the A and P type of virus was evident, immunization with pBSC/gag seemed to be slightly effective against FV-P only. Mice declared as protected were free of erythroblastoid infiltration in their spleen, liver and lungs, and the weight of all these organs was within normal limits.

Second immunization experiment

The experiment was repeated using the same plasmids, but only intradermal inoculation of DNA and the FV-P virus for challenge were used. For control purposes immunization with irradiated FLC 3cl8 and FLC IFN-α cl11 cells and non-irradiated FLC IFN-α cl11 cells were performed. Ten mice were included in each group. Three mice inoculated with non-irradiated FLC IFN-α cl11 cells developed intraabdominal ascitic tumours after the first inoculation; this might have been due to the appearance of rare revertants because the last three passages of these cells prior to immunization were performed in the absence of G418 (see Material and Methods). These three animals were eliminated from the experiment. Again, all mice were challenged with approximately 20 ELID50. The results are shown in Table 2. The disease developed in all untreated mice and in all but one animal treated with the control plasmid. The immunization effect was even more marked than in the first experiment. Eighty per cent of mice immunized with either pBSC/env or pBSC/gag were protected.

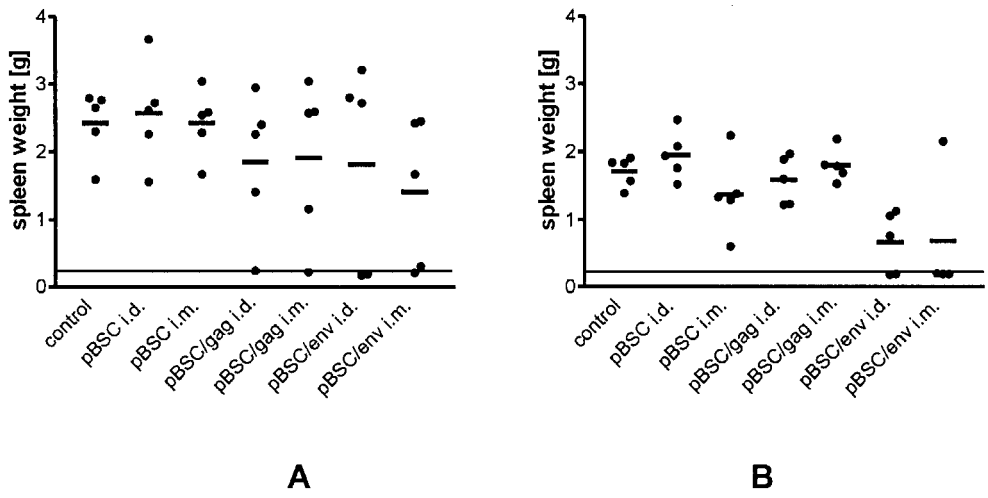

Fig. 3. The first immunization experiment – weight of spleens of mice challenged with the P type virus (A) and the A type virus (B). Mice were injected with three doses of plasmids given either intramuscularly (100 μg/dose) or intradermally (1 μg/dose). The thinline marks the maximum weight of normal spleen in DBA/2 mice. Short thick bars indicate the average weight of spleens in each group.

Serological response to vaccination and its relationship to protection to challenge

A proportion of immunized mice possessed the anti-Env and/or anti-Gag antibodies at the time of challenge. It was of interest to find out whether there was a relationship between the antibody presence and the protection to challenge. The results of this analysis are shown in Tables 3 and 4. Although the protection was seen somewhat more frequently in Env- and Gag-antibody-positive animals, the difference was quite small. In this respect it is noteworthy that in the groups immunized by the plasmids all those who were protected were free of detectable Env or Gag antibody at the challenge; on the other hand, the only two antibody-positive animals were not protected. These findings demonstrate a lack of association between the antibody presence and the protective immunity. It may also be of interest that after challenge, at the end of the observation period, no striking differences were detected in the occurrence of antibodies in the successfully immunized mice and those who developed the malignancy (results not shown).

To further examine the nature of the antibodies developed, the neutralization test with the sera either possessing or not possessing antibodies at the time of challenge was performed. No neutralization activity was observed. All animals developed the disease, and its course and pathological findings did not differ among the different groups of animals (results not shown).

Discussion

The FV model has repeatedly been used to elucidate the mechanisms involved in protective immunity against retrovirus-induced diseases. Several types of vaccine have been successfully employed to prevent FV-induced erythroleukaemia in mice. Correlation between protection and antibody development, CTL response and lymphocyte proliferation has been reported (for review see Hasenkrug and Chesebro, 1997), suggesting that both humoral and cell-mediated immune reactions are involved. Immunoprotective determinants have been identified in both the Env and Gag proteins and some evidence has been obtained that these determinants induce immune responses that interfere with different steps of the pathogenetic process (Ruan and Lilly, 1991; Hasenkrug et al., 1997; Uenishi et al., 1998). This has suggested that reactivity against a multiplicity of epitopes is needed to induce a full protection. It is noteworthy that immunization with the Gag protein has been reported in mouse strains in which immunization with the Env protein failed (Ishizara et al., 1991), which in its turn underlines the importance of genetic factors in the development of FV-induced disease and in protection from it.

It was not the purpose of the present experiments to analyse the different parameters of immunity against FV, but simply to explore whether efficient immunization by DNA vaccines against FV-induced leukaemia was possible. The results indicated that protective immunity in DBA/2 mice was indeed achieved by their immunization with DNA copies of viral genes. Both *env*- and *gag*-based vaccines were effective and the protective effect attained was comparable to that observed after immunization with continuous FV-transformed cell lines.

Although originally not planned for the present series of experiments, we could not resist the temptation

Table 3. Presence of anti-Env antibody and protection to challenge with FV

Group	No. of animals	No. of animals with *env* antibody before challenge	Protection in	
			env + antibody animals	*env* – antibody animals
pBSC/env	10	1/10	0/1	8/9
FLC 3cl8* (irradiated)	10	4/10	2/4	1/6
FLC IFN-α cl11* (irradiated)	10	1/10	1/1	7/9
FLC IFN-α cl11	7	7/7	7/7	-
All	37	13/37 (35.1%)	10/13 (76.9%)	16/24 (66.7%)

Table 4. Presence of anti-Gag antibody and protection to challenge with FV

Group	No. of animals	No. of animals with *gag* antibody before challenge	Protection in	
			gag + antibody animals	*gag* – antibody animals
pBSC/gag	10	1/10	0/1	8/9
FLC 3cl8* (irradiated)	10	7/10	3/7	0/3
FLC IFN-α cl11* (irradiated)	10	4/10	4/4	4/6
FLC IFN-α cl11	7	7/7	7/7	-
All	37	19/37 (51.4%)	14/19 (73.3%)	12/18 (66.6%)