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Immunity and vaccine control of *Echinococcus granulosus* infection in animal intermediate hosts

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Abstract. Much progress has been made with characterisation of the EG95 vaccine which can be used to prevent hydatid infection in animal intermediate hosts of *Echinococcus granulosus*. The vaccine comprises a single recombinant oncosphere antigen and the adjuvant Quil A. It induces complement-fixing antibodies that kill the invading oncosphere early in an infection. In the majority of vaccinated animals, no hydatid cysts occur following a challenge infection. However, a small number of viable cysts may occur in some vaccinated animals. The vaccine has proved effective in vaccine trials carried out in sheep in New Zealand, Australia, Argentina, Chile and China as well as in goats and cattle. Investigations of the genetic diversity of the gene encoding EG95 have identified no unequivocal variation within the G1 strain parasites; however DNA sequence diversity within the EG95 family of genes has been found in G6/G7 parasites. GMP production scale-up of the vaccine has been undertaken in New Zealand and China and it is expected that the vaccine will be become available through these sources for implementation as part of hydatid control programs worldwide.

Since its first description in 1996, the EG95 hydatid vaccine has proven highly effective and reliable in inducing protection against challenge infection with *Echinococcus granulosus* in a number of species of host and in several different countries (Lightowlers *et al.*, 1996a; Lightowlers *et al.*, 1999; Heath *et al.*, 2003).

Sufficient evidence has been gathered to indicate that the vaccine has great potential for practical use to reduce the level of transmission of *E. granulosus* and, indirectly, reduce the burden of hydatid disease on human health. Efforts are now being directed towards the scaling-up of vaccine production such that adequate quantities of quality-controlled vaccine are available for practical use.

The following discussion examines the background to development of the vaccine, investigations that have been undertaken to date and future prospects for implementation of vaccination in hydatid control programs.

Historical aspects

During the 1960's Michael Gemmell undertook many seminal scientific investigations on the immunology of *E. granulosus* and other taeniid cestode infections in sheep (Gemmell, 1962, 1964, 1966, 1967; Gemmell and Soulsby, 1968). He found using *Taenia ovis* that oncospheres injected into the tissues of sheep were capable of causing a viable metacestode infection and that this infection could be differentiated from an infection acquired by ingestion of eggs orally (Gemmell, 1962). Sheep that had been exposed in this way to an initial infection were immune to reinfection, but only if the initial infection used living, not dead, oncospheres. These data led to the hypothesis that protection was only elicited by exposure to the living parasite. However this hypothesis was inconsistent with earlier work undertaken with taeniid cestode parasites of laboratory animals (Miller, 1931; Miller, 1932; Kan, 1934; Campbell, 1936; Campbell, 1938). Subsequently, Rickard and his colleagues showed that protection could be demonstrated in rabbits and rodents to *Taenia* infections following the growth of their larval stages in intraperitoneal implants having a pore size of 0.22µm (Rickard and Bell, 1971a), indicating that the protective antigens were elaborated by the living parasites but that protection did not require direct exposure to the living organisms *per se*. Following the successful demonstration of *in vitro* culture of cestode larve from the oncosphere (Heath and Smyth, 1970; Heath, 1973a,b), before long it had been shown that the supernatants from oncospheres cultured in vitro could be used to induce protection against *Taenia* infections (Rickard and Bell, 1971b; Heath 1973a,b; Rickard and Adolph, 1976; Rickard and Adolph, 1977). It was recognised later that oncospheres were a potent source of host-protective antigens that could be demonstrated to be present in crude, non-living extracts of the parasites and without any requirement for culture *in vitro* (Rajasekariah *et al.*, 1980a, b; Osborn *et al.*, 1981; Rajasekariah *et al.*, 1982, 1985). Many of the host-parasite immunological characteristics of *E. granulosus* mirror those seen in other taeniid cestodes (Rickard and Williams, 1982; Heath, 1995) and, given these similarities, it was not surprising that oncospheres were found to be a rich source of host-protective antigens from *E. granulosus* (Osborn and Heath, 1982).

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The demonstration of high levels of protection against *Taenia* infection using a non-living source of antigens paved the way for development of practical vaccines. For vaccination of large numbers of livestock, practical quantities of *Taenia* larva cannot be propagated *in vitro* and so production of sufficient amounts of vaccine antigens required the advent and application of recombinant DNA technology. In the first successful application of this technology in the field of parasitology, a recombinant vaccine against *T. ovis* infection in sheep was described in 1989 (Johnson *et al.*, 1989). Close homologues of these host-protective antigens were found in to be expressed *Taenia saginata* oncospheres, and these homologous recombinant proteins were shown the be effective when used as vaccines to prevent cysticercosis in cattle due to *T. saginata* infection (Lightowlers *et al.*, 1996b). Homologues of the genes encoding host-protective oncosphere antigens of *T. ovis* and *T. saginata* were not evident in the genome of *E. granulosus* and so the strategy that had been used successfully for *T. saginata* could not be adopted for development of a vaccine against hydatid disease.

Development of the EG95 vaccine against hydatid disease

While the lack of homology between the host-protective oncosphere antigens of *Taenia* and of *E. granulosus* represented a set-back to rapid development of a vaccine against hydatid disease, the known presence of highly protective antigens in *E. granulosus* oncospheres (Osborn and Heath, 1982) indicated that a similar strategy to that used for *T. ovis* for identification and cloning of protective antigens would be appropriate also for *E. granulosus*. Heath and Lawrence (1996) described the intensive investigations that were undertaken and which identified individual host-protective proteins from *E. granulosus.* Their studies also raised antibodies that were specific for particular antigens or sub-sets of antigens and these were then used to identify antigens cloned from oncosphere mRNA (Lightowlers *et al.*, 1996a). One antigen clone, designated EG95, was found to express a protein that was capable of inducing protection against *E. granulosus* infection in vaccinated sheep.

EG95 vaccine proves reliable in different countries and in different animal species

Vaccine trials have now been carried in sheep against experimental challenge infection with *E. granulosus* in New Zealand, Australia, Argentina and Chile (Lightowlers *et al.*, 1999; Heath *et al.*, 2003) and on each occasion the vaccine has induced a high level of protection (Table 1). While some vaccinated animals were found to harbour hydatid cysts after the challenge infection, their number was reduced by approximately 99%, and half of the vaccinated animals were found to harbour no hydatid cysts. Heath *et al.* (2003) describe the levels of protection afforded by the EG95 vaccine against hydatid disease in cattle, where protection levels have been observed between 89 and 99%.

Duration of protection and field trials

Vaccine trials in sheep carried out in Argentina and China have determined that two immunisations affords protection lasting at least a year and that a third booster injection given after six - twelve months leads to solid immunity in sheep challenged 12 months or more after their first injection (Heath *et al.*, 2003). Field trials of the EG95 vaccine have been carried out over a number of years in the Province of Xinjiang, China (Heath *et al.*, 2003). The vaccine has proved highly effective, with annual vaccine booster injections maintaining protective immunity in field animals over a period of five years.

Vaccine scale-up

Substantial research has been undertaken in New Zealand into optimizing and scaling up GMP-quality manufacture of the EG95 vaccine. The vaccine production process have been adapted substantially from the affinity purification methodology used initially to prepare vaccine antigen (Lightowlers *et al.*, 1996a). This technology was not easily adapted to cost-effective commercial production and new methodologies were required to meet adequate levels of vaccine production and cost. These processes are currently being applied in a purpose-built GMP vaccine production unit in Beijing. Production validation runs are in progress in the plant and it is anticipated that this will provide vaccine which will allow practical application of the vaccine in hydatid control campaigns wherever this is desired.

Studies on genetic variability in EG95

The gene encoding EG95 has been found to be a member of a small multi-gene family (Chow *et al.*, 2001). This fact has served to complicate the study of genetic variability in the gene encoding EG95 in different parasite isolates and *E. granulosus* strains. Complications arise because of the need to clearly differentiate gene sequences between those that represent EG95 *per se* from those of other members of the closely related gene family. There are seven genes in the genome of the G1 strain parasite isolate from which the EG95 mRNA was isolated, six of which are expressed (Figure 1; Chow *et al.*, 2001). All members of the gene family were isolated and sequenced, providing information that has allowed the development of gene-specific primers which now enable gene-specific investigations to be undertaken (Chow *et al.*, 2004). Zhang *et al.* (2003) have published some information concerning genetic variability in the EG95 gene sequences from Chinese isolates of *E. granulosus*. Unfortunately, the PCR-generated gene sequences determined by Zhang *et al.* (2003) are impossible to interpret because the RT-PCR primers used in these studies could not exclude the production of heteroduplex PCR artefacts arising from the simultaneous amplification of several closely related target sequences (Zylstra *et al.*, 1998). Nor did their PCR primers allow any discrimination between the EG95 gene and other *eg*95 gene family members.

Table 1. Summary of EG95 vaccine trial results against an experimental challenge infection with *E. granulosus.*

Group	Number of cysts in individual sheep													Mean	Protection * %
Trial 1															
Controls	V**	85	49	39	11	0								36.8	
	NV	0	2	0	0	0								0.4	
EG95 Vaccinated	V	0	0	0	0	0								0	100
	NV	1	0	0	0	0								0.2	
Trial 2															
Controls	V	16	9	9	2	2	2	1	1	0				4.7	
	NV	25	14	8	12	6	4	45	7	4				13.9	
EG95 Vaccinated	V	1	1	0	0	0	0	0	0	0	0			0.2	96
	NV	4	1	4	3	2	1	0	0	0	0			1.5	
Trial 3															
Controls	V	64	62	51	23	11	7	4	4	3	2			23.1	
	NV	0	1	1	1	5	3	1	0	9	0			2.1	
EG95 Vaccinated	V	1	0	0	0	0	0	0						0.1	99
	NV	2	1	1	4	0	0	0						1.1	
Trial 4															
Controls	T	165	40	30	15	10	9	8	7	3	2			28.9	
EG95 Vaccinated	T	2	1	0	0	0	0	0	0	0	0	0	0	0.1	99

* Calculated on the number of viable cysts expressed as a percentage reduction in the mean number of cysts in vaccinated sheep compared with the mean number in control animals. ** V, viable cysts; NV, non-viable cysts; T, total cysts. Sheep were vaccinated with 50 µg protein plus 1 mg Quil A twice, one month apart, and challenged with *E. granulosus* eggs from parasites experimentally maintained in sheep and dogs in New Zealand (Trial 1), from a naturally infected Australian dingo (dingo/wallaby cycle; Trial 2), from a naturally infected Argentinian farm dog (dog/sheep cycle; Trial 3) and from a naturally-infected Chilian dog (dog/sheep cycle; Trial 4). Levels of protection were assessed 12-14 months after experimental infection (data from Lightowlers *et al.*, (1996a and 1999) except Trial 4, Drs Luis Rubilar and David Heath, unpublished observations).

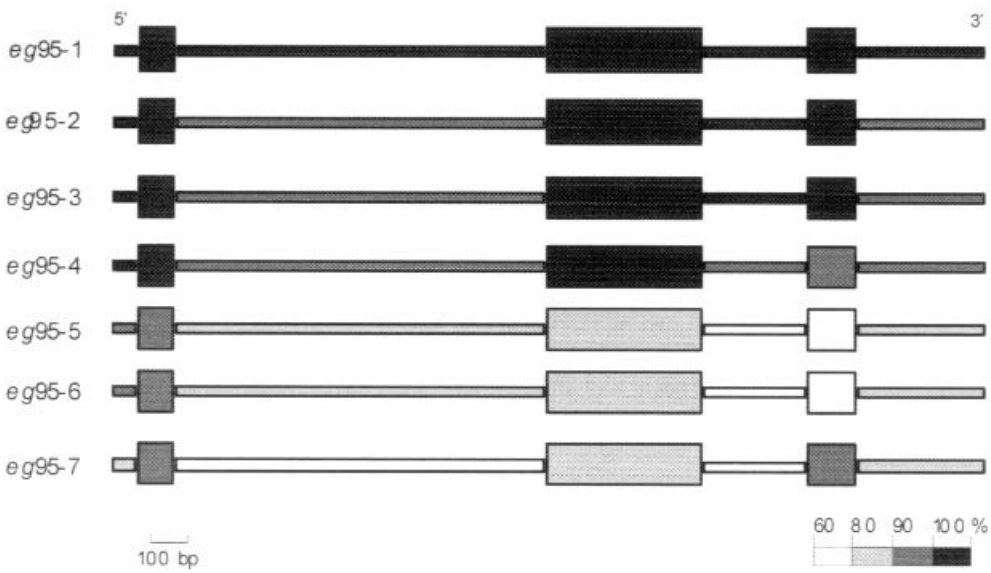

Fig. 1. Schematic representation of the EG95 family of genes from a G1 genotype isolate (the same isolate from which the EG95 mRNA was originally isolated). Seven genes were identified in the genome, all of which are expressed except the pseudogene EG95-7 (after Chow *et al.*, 2001).

The gene found to encode the EG95 vaccine protein corresponds to the expressed product of the gene designated *eg*95-1 (Chow *et al.*, 2001). However three other genes (*eg*95-2, *eg*95-3, *eg*95-4) express a protein identical to EG95 in the oncosphere of *E. granulosus.* Hence, the vaccine based on the expressed product of one gere would target the product of at least four genes. Two other genes in the EG95 gene family are also expressed in oncospheres. The proteins expressed by these genes are significantly different to EG95 and preliminary evidence suggests that these proteins are not host-protective (Heath, Gauci and Lightowlers, unpublished observations). Gene-specific investigations of genetic variability in *E. granulosus* with respect to the *eg*95-1 gene have begun (Chow, Gauci, Cowman and Lightowlers, unpublished observations) and, to date, no variation has been found in the predicted protein encoded by EG96 in any G1 genotype parasite examined to date. However significant variation has been found with G6/G7 genotype parasites. The potential impact this may have on the effectiveness of the EG95 vaccine for G6/G7 parasites remains to be determined.

Host-protective immune mechanisms induced by the EG95 vaccine

Two lines of evidence indicate that antibody and complement-mediated lysis of the invading oncosphere is a major host-protective mechanism induced by vaccination with the EG95 vaccine. Lambs born from EG95-vaccinated ewes are immune to *E. granulosus* challenge infection for the first few weeks of life (Heath *et al.*, 2003) due to the presence of specific anti-EG95 antibodies received from their mothers via colostrum. Secondly, it is possible to demonstrate the killing of *E. granulosus* oncospheres by antibody raised against EG95 together with complement (Heath and Lawrence, 1981; Heath *et al.*, 1981; Heath *et al.*, 1994; Woollard *et al.*, 1998; 1999). Oncosphere killing can be demonstrated by maintaining hatched and activated oncospheres in culture media together with heat-inactivated test sera and a source of complement. Sera from animals vaccinated with EG95 cause the death of oncospheres in the cultures whereas the presence of sera from control sheep do not prevent the survival and development of the cultured parasites. While this information does not exclude other host-protective immune mechanisms, it does indicate that specific antibody alone is sufficient to account for the host-protective effects of the EG95 vaccine.

Practical application of the EG95 vaccine

Heath *et al.* (2003) outlined the most effective procedure for use of the EG95 vaccine to prevent *E. granulosus* infection in livestock animals. Two immunisations are recommended in young animals, one month apart. The number of times each year that vaccinations would be given would depend on the local animal husbandry conditions. Where stock were borne over a single breeding season, only one vaccination period would be required per year (ie one vaccination period in which two vaccinations are given one month apart). In areas where stock are borne during two breeding seasons, two vaccination periods would be required because of the necessity to vaccinate young animals and ensure that they remain susceptible to infection for the shortest period practicable. Optimal levels of protection would be achieved if each of the young received both a primary as well as a booster immunisation within a short period of birth. However significantly enhanced protection could be expected if stock received a booster immunisation six-twelve months after the primary injections. Vaccine safety trials have been carried out in New Zealand, including investigations of safety in pregnant sheep and cattle. No adverse effects have been observed. Immunity persists after two injections for at least 12 months and longer term immunity can be achieved following a booster immunization given 6 or 12 months after the first two injections. Significant levels of protection can be transferred to lambs and calves with specific colostral antibody. In two large scale field trials in the Provinces of Qinghai and Xinjiang of China no adverse effects have been noted in the vaccinated animals and the vaccine has been highly effective in reducing naturally-acquired *E. granulosus* infection.

Preliminary investigations have indicated that the hydatid vaccine can be successfully delivered as a single immunisation mixed with multi-component clostridial vaccines, or mixed with an injectable anthelmintic. Incorporation of the hydatid vaccine as a component of other vaccines would provide an economic incentive to livestock owners to use such a vaccine while at the same time reducing the transmission of a medically important pathogen.

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References

Campbell DH (1936). Active immunization of albino rats with protein fractions from *Taenia taeniaeformis* and its larval form *Cysticercus fasciolaris*. American Journal of Hygiene 23: 104-113.

Campbell DH (1938). The specific absorbability of protective antibodies against *Cysticercus crassicollis* in rats and *C. pisiformis* in rabbits from infected and artificially immunized animals. Journal of Immunology 35: 205-216.

Chow C, Gauci CG, Cowman AF, Lightowlers MW (2001). A gene family expressing a host-protective antigen of *Echinococcus granulosus*. Molecular and Biochemical Parasitology 118(1): 83-88.

Chow C, Gauci C G, Cowman A F, Lightowlers M W (2004). *Echinococcus granulosus*: oncosphere-specific transcription of genes encoding a host-protective antigen. Experimental Parasitology, In press.

Gemmell MA (1962). Natural and acquired immunity factors inhibiting penetration of some haxacanth embryos through the intestinal barrier. Nature 194: 701-702.

Gemmell MA (1964). Species specificity of the immunogenic

complexes of the tapeworm hexacanth embryo. Nature 204: 705-707.

Gemmell MA (1966). Immunological responses of the mammalian host against tapeworm infections. IV. Species specificity of hexacanth embryos in protecting sheep against Echinococcus granulosus. Immunology 11: 325-335.

Gemmell MA (1967). Species specific and cross-protective functional antigens of the tapeworm embryo. Nature 213: 500-501.

Gemmell MA, Soulsby EJ (1968). The development of acquired immunity to tapeworms and progress towards active immunization, with special reference to Echinococcus spp. Bulletin of the .World Health Organization 39: 45-55.

Heath DD, Smyth JD (1970). *In vitro* cultivation of *Echinococcus granulosus, Taenia hydatigena, T. ovis, T. pisiformis* and *T. serialis* from oncosphere to cystic larva. Parasitology 61: 329-343

Heath DD (1973a). An improved technique for the in vitro culture of taeniid larvae. International Journal for Parasitology 3: 481-484.

Heath DD (1973b). Resistance to *Taenia pisiformis* larvae in rabbits. I. Examination of the antigenically protective phase of larval development. International Journal for Parasitology 3: 485-489.

Heath DD (1973c). Resistance to *Taenia pisiformis* larvae in rabbits. II. Temporal relationships and the development phase affected. International Journal for Parasitology 3: 491-497.

Heath DD, Lawrence SB (1981). *Echinococcus granulosus* cysts: early development in vitro in the presence of serum from infected sheep. International Journal for Parasitology 11: 261-266.

Heath DD, Parmeter SN, Osborn PJ, Lawrence SB (1981). Resistance to *Echinococcus granulosus* infection in lambs. Journal of Parasitology 67 :797-799.

Heath DD, Holcman B, Shaw RJ (1994). *Echinococcus granulosus*: the mechanism of oncosphere lysis by sheep complement and antibody. International Journal for Parasitology 24: 929-935.

Heath DD, Lawrence SB (1996). Antigenic polypeptides of *Echinococcus granulosus* oncospheres and definition of protective molecules. Parasite Immunology 18(7): 347-357.

Heath DD (1995). Immunology of *Echinococcus* infections. In: R.C.A. Thompson and A.J. Lymbery (Editors), *Echinococcus* and hydatid disease. CAB International, Wallingford, pp. 183-200.

Heath DD, Jensen O, Lightowlers MW (2003). Progress in control of hydatidosis using vaccination—a review of formulation and delivery of the vaccine and recommendations for practical use in control programmes. Acta Tropica 85: 133-143.

Johnson KS, Harrison GBL, Lightowlers MW, O'Hoy KL, Dempster RP, Lawrence SB, Vinton JG, Heath DD, Rickard MD (1989). Vaccination against ovine cysticercosis using a defined recombinant antigen. Nature 338: 585-587.

Kan K (1934). Immunological studies of *Cysticercus fasciolaris*. Keio Igaka 14: 663-687.

Lightowlers MW, Jensen O, Fernandez E, Iriarte JA, Woollard DJ, Gauci CG, Jenkins DJ, Heath DD (1999). Vaccination trials in Australia and Argentina confirm the effectiveness of the EG95 hydatid vaccine in sheep. International Journal for Parasitology 29: 531-534.

Lightowlers MW, Lawrence SB, Gauci C, Young J, Ralston M, Mass D, Heath DD (1996a). Vaccination against hydatidosis using a defined recombinant antigen. Parasite Immunology 18: 457-462.

Lightowlers MW, Rolfe R, Gauci CG (1996b). *Taenia saginata*: vaccination against cysticercosis in cattle with recombinant oncosphere antigens. Experimental Parasitology 84: 330-338.

Miller HM (1931). The production of artificial immunity in the albino rat to a metazoan parasite. Journal of Preventive Medicine 5: 429-452.

Miller HM (1932). Acquired immunity against a metazoan parasite by use of non-specific worm material. Proceedings of the Society for Experimental Biology 29: 1125-1126.

Osborn PJ, Heath DD (1982). Immunisation of lambs against *Echinococcus granulosus* using antigens obtained by incubation of oncospheres *in vitro*. Research in Veterinary Science 33: 132-133.

Osborn PJ, Heath DD, Parmeter SN (1981). Immunisation of lambs against infection with *Taenia ovis* using an extract of *Taenia ovis* eggs. Research in Veterinary Science 31: 90-92.

Rajasekariah GR, Mitchell GF, Rickard MD (1980a). *Taenia taeniaeformis* in mice: protective immunization with oncospheres and their products. International Journal for Parasitology 10: 155-160.

Rajasekariah GR, Rickard MD, Mitchell GF (1980b). Immunization of mice against infection with *Taenia taeniaeformis* using various antigens prepared from eggs, oncospheres, developing larvae and strobilocerci. International Journal for Parasitology 10: 315-324.

Rajasekariah GR, Rickard MD, Mitchell GF, Anders RF (1982). Immunization of mice against *Taenia taeniaeformis* using solubilized oncospheral antigens. International Journal for Parasitology 12: 111-116.

Rajasekariah GR, Rickard MD, O'Donnell IJ (1985). *Taenia pisiformis*: protective immunization of rabbits with solubilized oncospheral antigens. Experimental Parasitology 59: 321-327.

Rickard MD, Adolph AJ (1976). Vaccination of calves against *Taenia saginata* infection using a "parasite-free" vaccine. Veterinary Parasitology: 389-392.

Rickard MD, Adolph AJ (1977). Vaccination of lambs against infection with *Taenia ovis* using antigens collected during short-term in vitro incubation of activated *T. ovis* oncospheres. Parasitology 75: 183-188.

Rickard MD, Bell KJ (1971a). Immunity produced against *Taenia ovis* and *T. taeniaeformis* infection in lambs and rats following *in vivo* growth of their larvae in filtration membrane diffusion chambers. Journal of Parasitology 57: 571-575.

Rickard MD, Bell KJ, (1971b). Successful vaccination of lambs against infection with *Taenia ovis* using antigens produced during *in vitro* cultivation of the larval stages. Research in Veterinary Science 12: 401-402.

Rickard MD, Williams JF (1982). Hydatidosis/cysticercosis: immune mechanisms and immunization against infection. Advances in Parasitology 21: 229-296.

Woollard DJ, Gauci CG, Heath DD, Lightowlers MW (1998). Epitope specificities and antibody responses to the EG95 hydatid vaccine. Parasite Immunology 20: 535-540.

Woollard DJ, Gauci CG, Lightowlers MW (1999). Synthetic peptides induce antibody against a host-protective antigen of *Echinococcus granulosus*. Vaccine 18: 785-794.

Zhang W et al. (2003). Short report: *Echinococcus granulosus* from Xinjiang, PR China: cDNAS encoding the EG95 vaccine antigen are expressed in different life cycle stages and are conserved in the oncosphere. American Journal of Tropical Medicine and Hygiene 68: 40-43.

Zylstra P, Rothenfluh HS, Weiller GF, Blanden RV, Steele EJ (1998). PCR amplification of murine immunoglobulin germline V genes: strategies for minimization of recombination artefacts. Immunology and Cell Biology 76: 395-405.