The recombinant plasmid DNA, 50 μg in 0.25 ml with ISA 50 adjuvant per chick and the other group without any adjuvant was inoculated intramuscularly in the thigh of three-day-old chicks. Another group of chicks was inoculated with vector alone and a group was kept as healthy control. All the chicks were free from IBD antibody as determined by serum neutralization (SN) test using sera samples. At 21 days post-vaccination, all the birds were challenged with 10,000 ID_{50} of virulent IBD virus per bird, intramuscularly. The birds were observed for 14 days for sign of disease or death.

VP2 gene was synthesized and cloned successfully in pUC29 and recloned in pVAXI (Figures 1–3). The recombinant plasmid containing the VP2 gene expressed the VP2 protein in CEF cell culture as detected by immunoperoxidase test. The recombinant plasmid when used as DNA vaccine in chicken was found to induce protective immunity (Table 1). It gave 100% protection in the chicken. The SN antibody titre was found to be 256 and 512 in vaccinated groups, while no antibody level was detected in vector alone and healthy control groups. The study corroborated the observations of previous workers[10], who found protection against the disease from insect cell-derived VP2 of IBD. The VP2 gene expressed in baculovirus recombinant plasmid was also found to protect the chickens from the disease[8]. About 55% protection was found by expressing IBD VP2 gene in recombinant Marek's disease virus and using it as vaccine[12]. In our study, 100% protection was observed, which showed that the DNA vaccine developed in the present study was highly effective.

It is evident from the present work that recombinant, plasmid DNA can be used as DNA vaccine against IBD. Since DNA vaccine needs no cold chain and it is cheaper to produce on a large scale, it can be widely used as an effective vaccine against IBD under Indian conditions.

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Isolation and characterization of a mouse embryonic stem cell line that contributes efficiently to the germ line

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Embryonic stem (ES) cells have proven vital for introduction of pre-defined genetic modifications into mouse germ line. Here, we describe the isolation of a mouse ES cell line namely, R1.9 through sub-cloning and karyotyping. R1.9 ES cells have normal chromosomal complement and contribute extensively to chimaerism and germ line. We have been successfully using R1.9 ES cells in our laboratory for creation of novel strains of mouse through gene targeting.

THE isolation and genetic manipulation of embryonic stem (ES) cells has been a major achievement in mammalian developmental biology with far-reaching implications in almost all areas of mammalian biology. Murine embryonic stem cells are pluripotent cells harvested from the inner cell mass of mouse blastocysts and grown *in-vitro* under conditions inhibiting their spontaneous differentiation[1,2]. ES cells are now widely used for introduction of genetic alterations into the mouse germ line. This is achieved in a number of stages. First, a targeting vector, containing the genetic modifications to be introduced as well as genes conferring drug resistance or sensitivity for selection is constructed. This targeting vector containing the desired mutation is electroporated into ES cells. In most cells, the targeting vector inserts randomly into the ES genome. However, in a few cells, the targeting vector pairs with the homologous sequences and transfers the mutation to the corresponding endogenous gene on the chromosome. Screening procedures are then used to identify the rare ES cell in which the targeted event has occurred. Next, the tar-

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geted ES cells integrated with pre-implantation stage embryos are surgically transferred into the uterus of a foster mother where development is allowed to term. The resulting animal is chimaeric, since it is composed of cells derived from both the donor stem cells and the host embryo. If the chimaerism extends to the germ line, breeding of chimaeras would result in pups carrying a mutated allele. Interbreeding of the heterozygous siblings yields animals homozygous for the desired mutation[3,4]. This is the method of choice for production of 'knock-out' animals to address the *in-vivo* functional significance of genes and to create mouse models of human diseases. One of the basic requirements for the successful application of this technology is to maintain ES cell lines in a state compatible with germ-cell formation[2,5]. In our efforts to establish ES cell technology at CCMB, we report here subcloning and characterization of a germ line competent ES cell line, namely, R1.9.

R1 ES cells, originally isolated from (129/SvJ × 129/Sv-CP) F 1 3.5 day mouse blastocyst, is known to contribute efficiently to the germ line[5,6]. The cells are heterozygous for the albino locus ($+/c^{-ch}$) and for the pink eye locus ($+/p$). Germ line transmission from ES component of chimaeras mated to an albino strain of mice would generate agouti and chinchilla pups.

We procured these cells at passage-13 and cultured them on feeder layers derived from mouse embryonic fibroblast according to standard protocols[7]. All cultures were monitored against mycoplasma contamination from time to time. While we could grow R1 cells regularly in our laboratory, repeated efforts to generate chimaeric mice did not meet with any success. Karyotyping of these cells revealed that more than 80% of the cells in our cultures were karyotypically abnormal. Chromosomal abnormalities in ES cells are not unknown and these may reflect suboptimal culture conditions. It is likely that a certain proportion of ES cells in a culture show abnormal chromosomal complement over prolonged *in-vitro* culture time. This abnormality confers some selective growth advantage to these cells, thereby reducing the germ line potential of the ES cell population as a whole[6,8]. To isolate and establish an ES cell line with germ line transmission, we proceeded to subclone R1 ES cell line. Briefly, R1 cells were plated and cultured at low density (2000 cells/100 cm^2). After one week, 18 individual ES cell clones were picked up, expanded and subjected to karyotype analysis. One of these clones showed consistently normal chromosome number and this was propagated as R1.9 ES cell line (Figure 1).

To test the pluripotency of R1.9 ES cells, we proceeded to generate chimaeric mice through co-aggregation of these cells with CD1 morulae[9]. For aggregation, 8–10 ES cell clumps obtained by trypsinising the ES cell culture, were sandwiched between two morulae in aggregation wells made by pressing a darning needle into the plastic bottom of a culture plate[9]. The aggregates were cultured overnight in micro drops of M16 media[7] and were transferred the following day into the uteri of 2.5 day pseudo pregnant recipient mice[7,9].

In each litter, chimaeric animals were obtained (Table 1) as revealed by the presence of the agouti coat colour

Figure 2. One of the chimaeras generated from the R1.9 ES cells.

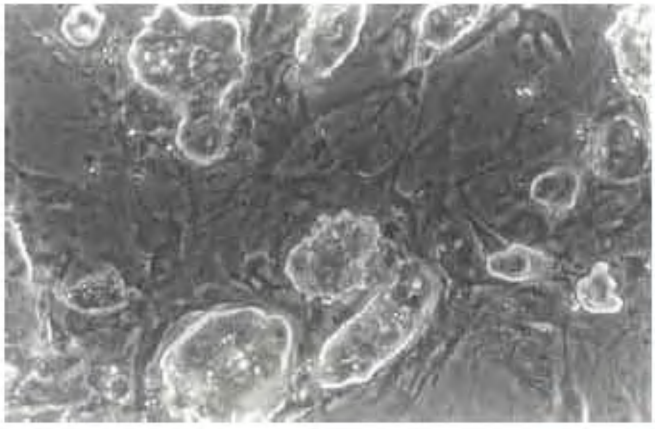

Figure 1. R1.9 embryonic stem cells on a feeder layer in culture.

Figure 3. Germ line transmission from one of the chimaeras: Agouti and chinchilla pups obtained by mating a chimaera indicates germ line transmission from R1.9 ES cells.

Table 1. Production of chimaeric mice from R1.9 ES cells

Technique	No. of manipulated embryos	No. of recipient females	Total pups born	No. of chimaeras	Sex ratio of chimaeras male : female
Morula aggregation (wild-type R1.9 cells)	65	5	14	6	5 : 1
Blastocyst injection (targeted R1.9 cells)	136	10	20	7	6 : 1

(from ES cells) in the background of albino coat component of CD1 host embryos (Figure 2). The chimaeras exhibited varying degree of ES cell contribution (15 to 95%) as judged from the extent of agouti coat colour. Sex ratio was skewed in favour of males (R1.9 is a male ES cell line) among the chimaeras as was expected after introduction of male ES cells into unsexed embryos. Breeding of the chimaeras resulted in progeny with the chinchilla and agouti coat colours (Figure 3) proving the germ line potency of the R1.9 ES cell line. Subsequently, we have used R1.9 ES cells to introduce targeted deletion of κ-casein gene. R1.9 clones mutated at this locus were injected into FVB/N or C57BL/6J blastocysts (Table 1). Blastocyst injection involved microinjection of 10–15 disaggregated ES cells into the blastocoel. The manipulated blastocysts were transferred without any further culture to the recipient females[7,10]. We obtained pups carrying the mutation. Three chimaeras, out of seven tested for germ line transmission, produced pups from the ES cell component. The efficiency of germ line transmission in these experiments (42.9%) based upon the number of chimaeras bred was better than that of original R1 ES cell (25.3%)[5]. R1.9 ES cell line, isolated and established in our culture conditions, is now routinely being used for inserting defined genetic modifications into mouse germ line.

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Age-dependent pollen abortion in cashew

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The effect of plant age on pollen abortion of *Anacardium occidentale* L., family Anacardiaceae, was tested. Using Alexander's stain, which differentiates aborted and non-aborted pollen grains, the proportion of aborted pollen versus plant age, size variation in aborted and non-aborted pollen grains, and number of pollen grains per flower were estimated. Aborted pollen was smaller than non-aborted pollen, regardless of plant age. The proportion of aborted pollen, varied from 22.5 to 46.8%, showing a steady increase with plant age, and the number of pollen grains per flower also increased with age. These results support the qualitative prediction that pollen abortion gradually increases with ageing, reflecting an increase in genetic load with age.

CASHEW, *Anacardium occidentale* L. (Anacardiaceae), is a hardy, drought-resistant, tropical or subtropical tree. It is distributed in tropical America, from Mexico and West Indies to Brazil and Peru[1]. Ranging from warm temperate moist to tropical very dry to wet forest life zones, cashew is reported[2] to tolerate annual precipitation of 7–42 dm, annual temperature of 21–28°C, and pH of 4.3–8.7. Globally, India is the leading producer; other countries producing cashew include Mozambique and Tanzania[3]. The demand for cashew is increasing, but the availability of seed is often a limiting factor for several reasons[4–6]. Consequently, an understanding of the factors affecting cashew seed production has impor-

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