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CD4 negative mice as a model for immunodeficiency

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SUMMARY

CD4 is a co-receptor required for T cell helper functions. A mouse strain without CD4 expression has been generated. These animals, surprisingly have a normal CTL response and reduced, but not absent, humoral responses. A new population of MHC class II restricted $CD4^-CD8^-Tcr\alpha\beta^+$ T cells has emerged which possess helper function potential. These findings have important implications on disease situations where CD4 cells are decreased or absent.

1. INTRODUCTION

The CD4 molecule is known to be involved in the process of MHC class II restricted antigen recognition by the T cell receptor (Schwartz 1985; Parnes 1989). The precise role of the CD4 molecule in antigen recognition is not well understood and the influence that it has on T cell development even less so.

T cell precursors entering the thymus express CD4 at a low level. They go through a stage in their development when the thymocytes express neither CD4 nor CD8 (double negative). They then express both CD4 and CD8 (double positive) and low levels of CD3 and TcR. It is at this stage of development that the T cells undergo positive and negative selection and eventually emerge as mature $CD4^+8^-$ or $CD4^-8^+$ (single positive) T cells. Preliminary evidence from studies using antibodies to the CD4 molecule suggests that the CD4 molecule is involved in the thymic selection of the T cell repertoire (Fowlkes *et al.* 1988; MacDonald *et al.* 1988). This role of the CD4 molecule would become more clear if we were to mutate the CD4 gene such that the T cells did not express the CD4 molecule on their surface. To this end, we have disrupted the CD4 gene in the pluoripotent embryonic stem (ES) cells (Evans & Kaufman 1981) through a targeted mutation by homologous recombination (Smithies *et al.* 1985; Thomas & Capecchi 1987). Germ-line transmission of the mutation has resulted in the generation of a mutant mouse strain that does not express the CD4 molecule on the cell surface. Surprisingly, this animal can mount cytotoxic T cell responses against viruses. Furthermore, Ig against specific viral antigens were also produced, albeit lower than normal animals.

2. GENE TARGETING OF CD4

The CD4 molecule is a 55 kDa cell surface glycoprotein found on a sub-population of lymphocytes, and exists on the cell surface as a monomeric structure. The CD4 gene in the mouse is 26 kilobase (kb) long and has ten exons and nine introns (Gorman *et al.* 1987). For homologous recombination, a replacement-type vector was designed to create a mutant CD4 gene. This was a 2.8 kb genomic fragment containing exon 5 and exon 6. Exon 5 was disrupted by insertion of the bacterial neomycin resistance gene. This DNA construct was introduced into D3 ES cells by electroporation. Eight independent lines of ES cells with one of the alleles at the CD4 locus disrupted were generated. Southern blot analysis confirmed the replacement of the endogenous CD4 gene by single copy insertions of the construct by homologous recombination. Six of these clones were injected into preimplantation embryos from C57B1/6J mice. Mice with germ-line transmission representing four of the six independent clones were obtained.

3. PHENOTYPIC ANALYSIS

Mice lacking surface expression of CD4 were healthy, fertile and indistinquishable from heterozygous or wild-type littermates on gross physical inspection. The number of cells in the lymphoid organs were similar to those in heterozygous or wild-type mice. Surface expression of CD4, however, was not detected on thymocytes or lymph node cells from mice homozygous for the mutant CD4 alleles. Staining of the thymocytes in the $CD4^-$ homozygous mice shows that $CD8^+$ cells are present in normal numbers in the thymus but expand in the periphery to occupy the compartment that would otherwise have been occupied by $CD4^+$ cells. Analysis using antibodies against CD3 and $TcR_{\alpha\beta}$ showed that the expression of these molecules is normal in thymocytes of the mutant mice. There is no difference in the proportion of cells with high and low levels of $TcR_{\alpha\beta}$ on the surface, between the wild-type mice, heterozygous mice and the homo-

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zygous mutant mice. This indicates normal maturation of the $CD8^+$ T cells, as far as the expression pattern of $TcR_{\alpha\beta}$ on the thymocytes is concerned. These results demonstrate that the expression of CD4 on the surface of the progenitor cells and on the double positive thymocytes is not required for the normal development of $CD8^+$ cells. The functional consequences of the $CD4^-$ phenotype are being assessed at present (Rahemtulla *et al.* 1991).

4. CYTOTOXIC T CELLS AGAINST VIRUSES

To test if the animals can mount cytotoxic T lymphocyte responses against viruses, the CD4 mutant mice were infected with lymphocytic cloriomeningitis virus (LCMV). Results show that they can successfully generate specific CTL against these viral antigens, as measured by their ability to lysis viral infected target cells infected with the same virus. Similarly, CD4 negative mice infected with Vaccinia viruses can also develope CTLs with specificity against target cells. These results indicate that mice lacking in expression of cell surface CD4 can successfully generate CTLs against viral antigens (Rahemtulla *et al.* 1991, 1993).

5. HUMORAL RESPONSES IN $CD4^-$ MICE

CD4 cells are believed to be essential for the process of Ig isotype class switching (Schwartz 1985; Parnes 1989). Thus we tested the ability of the CD4 negative mice to mount specific IgM and IgG responses against antigens. When these animals are challenged with an antigen (e.g. sheep red blood cells) or a virus (e.g. vesticular stomatitis virus VSV), the IgM responses are similar to those of mice with $CD4^+$ cells, indicating that there is no difference between normal and mutant animals. However, when the level of IgG specific for the antigens was monitored, the levels of specific antibodies against these antigens was found to be lower (about 5–50-fold, depending on the doses of antigens). Thus it appears that, although the ability to mediate Ig isotype class-switching is impaired in animals without CD4 cells, the ability to induce this function is none the less present in mutant animals. This is an exception to the paradigm that Ig isotype class-switching requires CD4 cells. This data indicates that a subpopulation of T cells is substituting for the role of $CD4^+$ lymphocytes.

6. THE PRESENCE OF $CD4^-CD8^-\alpha\beta^+$ CELLS

In the absence of $CD4^+$ cells, a subset of T lymphocytes capable of performing the functions of helper cells have been generated. With the use of antibodies specific for CD8, CD4, Thy.1 and $TcR\alpha\beta$, we determine by *in vivo* depletion studies, that the subpopulation of lymphocytes mediating these biological functions had a phenotype of $CD4^-CD8^-TcR\alpha\beta^+$. Using FACS analysis cells with this phenotype can be found at a level of about 10% of total T cells in the $CD4^-$ mice (Rahemtulla *et al.* 1993).

7. CONCLUSION

We have generated a mouse strain that does not express CD4 cells by homologous recombination in embryonic stem cells. Surprisingly, we found that this animal can perform T cell functions including generating T killer cells and T helper cells. A new population of T cells with a phenotype of MHC class II restricted $CD4^-CD8^-TcR^{\alpha\beta+}$ emerged to substitute for the $CD4^+$ cells. This phenomenon may have physiological implications for clinical situations in which CD4 cells are reduced or absent.

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