antigen in Muc1-transgenic mice[9]. Moreover, there has been no evidence of normal tissue toxicity from the DC fusion cell vaccines.

Kugler and colleagues have now developed a therapeutic vaccine for patients with metastatic renal cell cancer by fusing DCs and autologous tumor cells with an electrical pulse. Response rates in the treatment of metastatic renal cell cancer with chemotherapeutic or hormonal agents are less than 10%. Disease regressions have also been demonstrated in only a minority of patients treated with interferons or interleukin-2. Here, the authors report tumor responses in seven of seventeen patients vaccinated with the fusion cells and prolonged stabilization of disease in two additional patients. Four patients achieved complete regression of their disease and have had no evidence of recurrence for periods of up to 21 months. In these patients, regressions were found in diverse metastatic sites and involved large tumor masses. Other than mild, transient fever, and pain at metastatic sites, there were no adverse effects. In addition, there was no evidence of autoimmune disease. As the authors point out, their provocative findings were obtained in a small cohort of patients and longer follow-up is needed to fully assess the effect of the vaccine. Nonetheless, the substantial regressions often seen within weeks of the first immunization indicate the potency of these vaccines and the responsiveness of the immune system to an adequate stimulus.

There are many experimental issues raised by the study of Kugler *et al.* that relate to the development of an optimal fusion cell vaccine. The authors used autologous tumor cells that had been fused to allogeneic DCs from random donors. Allogeneic stimulation of autologous T cells could contribute to induction of the immune response to the tumor (Fig. 1). In contrast, fusions with autologous DCs may be more effective in targeting tumor cells with downregulated expression of MHC molecules (Fig. 1). Fusion cell dosages and the number of vaccinations required represent additional variables that will need to be optimized in future studies. In cases of a mixed response or progressive disease, fusions with the different tumor metastases, which could be clonal, may also be required to achieve a complete response. Additionally, it will be important to determine whether fusion cell vaccines can induce immunity against multiple tumor antigens, and prevent tumor cell escape by antigen downregulation. It will also be important to identify the tumor antigens that best stimulate a potent anti-tumor response. Kugler *et al.* have already begun to address this issue by demonstrating that the fusion cell vaccine induces CTL activity against the Muc1 antigen, which is widely overexpressed in breast and other carcinomas[10].

If confirmed, the results of this study represent an unprecedented advance in the selective and non-toxic immunotherapy of a disseminated and lethal carcinoma. In addition to the potential benefits for patients with metastatic renal cell cancer, the findings should provide the impetus for assessing effectiveness of fusion cell vaccines in the treatment of other tumors. The immunotherapy of renal cell or other cancers with fusion cell vaccines could contribute to fulfilling the promise that emanated from the work of Jenner and has been anticipated from the success of vaccines against smallpox, polio and other infectious diseases.

1. Kugler, A. *et al.* Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. *Nature Med.* **6**, 332–336 (2000).
2. Gong, J., Chen, D. & Kufe, D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. *Nature Med.* **3**, 558–561 (1997).
3. Benacerraf, B. Role of MHC gene products in immune regulation. *Science* **212**, 1229–1238 (1981).
4. Young, J. W. & Steinman, R. M. The hematopoietic development of dendritic cells: a distinct pathway for myeloid differentiation, *Stem Cells* **14**, 376–387 (1996).
5. Banchereau, J. & Steinman, R. M. Dendritic cells and the control of immunity. *Nature* **392**, 245–252 (1998).
6. Kohler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495 (1975).
7. Guo, Y. *et al.* Effective tumor vaccine generated by fusion of hepatoma cells with activated B cells. *Science* **263**, 518–520 (1994).
8. Stuhler, G. & Walden, P. Recruitment of helper T cells for induction of tumor rejection by cytolytic T lymphocytes. *Cancer Immunol. Immunother.* **39**, 342–345 (1994).
9. Gong, J. *et al.* Reversal of tolerance to human MUC1 antigen in MUC1 transgenic mice immunized with fusions of dendritic and carcinoma cells. *Proc. Natl. Acad. Sci. USA* **95**, 6279–6283 (1998).
10. Kufe, D. *et al.* Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma* **3**, 223–232 (1984).

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Sane genetics for schizophrenia

Although past epidemiological studies have supported the theory that there is a genetic component to schizophrenia, the genetic data have been inconsistent. However, an overall analysis indicates several chromosome regions with good candidate genes for schizophrenia susceptibility.

BRIEN RILEY[1] & ROBERT WILLIAMSON[2]

DESPITE GREAT RESISTANCE to the theory that there is a genetic basis for psychiatric disease, epidemiological studies over the past 30 years have consistently demonstrated that genetic factors are important in the etiology of schizophrenia. Repeated analyses of family, adoption and twin data sets suggest a 10-fold increase in lifetime risk for relatives of schizophrenics. Additionally, 'adopted-away' children have the same risk as their biological (rather than their environmental) families, and concordance among monozygotic twins is approximately 50% (ref.1).

Twin studies also show that susceptibility to schizophrenia has a non-genetic component—monozygotic twins are not 100% concordant, and dizygotic twins (genetic siblings) have about twice the risk of developing schizophrenia of ordinary siblings. Analysis of concordance in first-, second- and third-degree relatives suggests that variants at three or more separate loci are required to confer susceptibility, and that these allelic variants increase risk in a multiplicative rather than additive manner, with the total risk being greater than the sum of the indi-

Table 1 Chromosome regions giving multiple positive results for schizophrenia susceptibility[7].

Chromosome	Number of findings	Sample source
1q32-q44	two plus breakpoint data	Finland, US (mixed), Scotland
2q11-q21	four	Austria, Germany, Israel, US, Australia
4q31	two	US (African-American), Finland
5q22-q31	two	Ireland, Germany
6p24-p22	three plus collaboration	Ireland, Germany, Israel
6q16-q22	three	US (mixed, two; African-American)
8p22-p21	three plus collaboration	US (mixed, three)
9qcen-q22	three	Germany, Israel, US (mixed), Australia, Finland
9q34.3	two	South Africa, US (African-American)
10p14-p13	three	US (Caucasian), Ireland, Germany
13q14-q32	five	Taiwan, US (mixed, two), UK, Iceland,
15q13-q14	three	US (Caucasian), US (African-American), South Africa
18p11	two	Germany, Israel, UK
22q12-q13	five plus collaboration	US (mixed), US (Caucasian), UK, Germany
Xp21-p11	four	UK, US (mixed), Finland

vidual risks conferred by each variant[2]. As schizophrenia is a common illness with lifetime risk of about 1% (ref. 3), the variants must be common, on the order of 14–20% in the general population[4].

Variations in diagnostic criteria and methods of genetic analysis have made it difficult to compare data sets between studies. The failure to replicate a finding may be due to ethnic differences in the frequency of a particular variant, to random variation in the inputs to liability present in two different samples, or to lack of power. The exclusion of an effect in a particular gene or chromosome region is nearly impossible, so negative data cannot be used to rule out possibilities. However, the situation is improving.

Although some high-profile findings, including the first reported schizophrenia linkage to chromosome 5 (ref. 5) and another on chromosome 3 (ref. 6), have never been replicated, analysis of the accumulated data indicates involvement of several different chromosome regions in susceptibility to schizophrenia[7] (Table I). For some examples, such as chromosome 6p24–22, collaborative studies have yielded positive net results, even though many of the individual data sets were negative, demonstrating the pitfalls of analyzing many small samples rather than a single, large one. Many other chromosome regions have also been implicated on more than one occasion in smaller studies.

Several of the loci suggested to be involved in schizophrenia are in regions containing genes required for normal neural development and function. One of these loci encodes the alpha-7 nicotinic acetylcholine receptor subunit (CHRNA7; 15q13–14), involved in nicotine binding and possibly in the very high incidence of smoking seen in schizophrenics. Sensory gating deficit, a secondary phenotype common in schizophrenics and rare in normal individuals, and normalized by both nicotine and clozapine, has also been linked to this gene[8].

Another secondary trait associated with schizophrenia, abnormal eye tracking, maps to 6p22–24(ref. 9), although the exact gene associated with this defect has not yet been identified. The velo-cardio-facial syndrome (VCFS) locus on chromosome 22, known to underlie a specific defect in craniofacial development, may also be a candidate for schizophrenia susceptibility: there is a subtle neurodevelopmental component to schizophrenia possibly involving defective neuronal migration[10]. Psychotic features are common in VCFS patients[11], and facial dysmorphology is common in schizophrenics[12].

Studies with mouse models also support the possibility of involvement of one of these loci. Mice lacking the NMDA receptor central subunit develop negative symptoms commonly associated with schizophrenia, such as withdrawal, poor social functioning and lack of volition[13]. The human chromosomal region containing this gene, 9q34.3, showed positive evidence for linkage to schizophrenia in two independent studies of African[4] and African-American[14] populations. In addition to findings on 9q34.3, there is considerable correspondence between the results of a genome screen of African-American families[14] and data obtained from South African Bantu families[4,15]. Analysis of founder populations (like the Bantu, in which a small, usually migrant, group with a limited gene pool gives rise to a large contemporary population) are useful in the study of schizophrenia genetics because they have higher frequencies of liability alleles at certain loci.

The feasibility of using single nucleotide polymorphism maps to link locus-specific mutations to disease has recently been shown for the non-insulin-dependent diabetes mellitus locus NIDDM1 (ref. 16). This study also developed useful new methods for detecting interactions between loci on separate chromosomes (showing that there is much greater input to the disease from one locus in the presence of input from the another). Although this technique falls short as a true multi-locus analysis, which would allow analysis of two or more loci simultaneously, it will provide a tool for gaining a better understanding the polygenic biology of a complex trait. Large screens for human mutations in candidate genes such as CHRNA7 and in non-coding regulatory regions are also underway.

In the long term, schizophrenia genome studies combined with functional studies in developmental biology and neurobiology will lead to a better understanding of the etiology of schizophrenia. Understanding the pathogenesis of schizophrenia will require that we initially identify at least one predisposing gene, perhaps CHRNA7, which will then allow us to begin to identify other interacting loci. Additional expression studies and the development of animal models will also be required. Research into the genetic basis of NIDDM1 has shown that these types of studies can begin before a full functional analysis of the gene product is made. Discovering the genetic basis of schizophrenia is likely to be slow, involving the analysis of many gene products. However, combined epidemiological, genetic, and functional genomic analyses are needed to provide real answers to this intractable and destructive medical mystery.

1. Kendler, K.S. & Diehl, S.R. The genetics of schizophrenia: A current, genetic-epidemiologic perspective. *Schizophr. Bull.* **19**, 261–285 (1993).
2. Risch, N. Linkage strategies for genetically complex traits. I. Multilocus models. *Am. J. Hum. Genet.* **46**, 222–228 (1990).
3. Gottesman, I. in *Schizophrenia Genesis* (W.H. Freeman, New York, 1991).
4. Riley, B.P. *et al.* A linkage study of the N-methyl-D-aspartate receptor subunit gene loci and schizophrenia in southern African Bantu-speaking

families. *Psychiatr. Genet.* **7**, 57–74 (1997).
5. Sherrington, R. *et al.* Localization of a susceptibility locus for schizophrenia on chromosome 5. *Nature* **336**, 164–167 (1988).
6. Pulver, A.E. *et al.* Schizophrenia: A genome scan targets chromosomes 3p and 8p as potential sites of susceptibility genes. *Am. J. Med. Genet. Neuropsychiatric Genet.* **60**, 252–260 (1995).
7. Riley, B.P. & McGuffin, P. Linkage and associated studies of schizophrenia. *Sem. Med. Genet.* (in press).
8. Freedman, R. *et al.* Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc. Natl. Acad. Sci. USA* **94**, 587–592 (1997).
9. Arolt, V. *et al.* Eye tracking dysfunction is a putative phenotypic susceptibility marker of schizophrenia and maps to a locus on chromosome 6p in families with multiple occurrence of the disease. *Am. J. Med. Genet. Neuropsychiatric Genet.* **67**, 564–579 (1996).
10. Jakob, K. & Beckmann, H. Prenatal developmental disturbances in the limbic allocortex in schizophrenics. *J. Neural Transm.* **65**, 303–326 (1986).
11. Murphy, K.C., Jones, L.A. & Owen, M.J. High rates of schizophrenia in adults with velocardial-facial syndrome. *Arch. Gen. Psychiatry* **56**, 940–945 (1999).
12. Bassett, A.S. & Chow, E.W.C. 22q11 deletion syndrome: A genetic subtype of schizophrenia. *Biol. Psychiatry* **46**, 882–891 (1999).
13. Mohn, A.R., Gainetdinov, R.R., Caron, M.G. & Koller, B.H. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* **98**, 427–436 (1999).
14. Kaufmann, C.A. *et al.* NIMH genetics initiative millennium schizophrenia consortium: Linkage analysis of African-American pedigrees. *Am. J. Med. Genet. Neuropsychiatric Genet.* **81**, 282–289 (1998).
15. Riley, B.P. *et al.* Haplotype transmission disequilibrium and evidence for linkage of the CHRNA7 gene region to schizophrenia in southern African Bantu families. *Am. J. Med. Genet. Neuropsychiatric Genet.* (in press).
16. Cox, N.J. *et al.* Loci on chromosomes 2 (NIDDM1) and 15 interact to increase susceptibility to diabetes in Mexican Americans. *Nature Genet.* **21**, 213–215 (1999).

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Pot of gold for glioma therapy

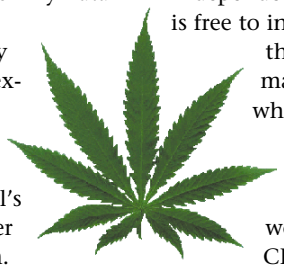
Cannabinoid agonists arrest tumor progression in a rodent model of malignant glioma. Will these molecules provide a starting point for new strategies for anti-cancer therapy (pages 313–319)?

DANIELE PIOMELLI

CANNABINOIDS HAVE HAD a long and interesting medical history. Soon after the British physician W. B. O'Shaugnessy had brought back from India an account of the remarkable effects of the cannabis plant, the medical communities in Europe and the US eagerly adopted it into their pharmacopeias. Cannabis, noted Robert Christison in his 1848 dispensatory, "promises to be an important article in Materia Medica…which deserves a more extensive enquiry than any hitherto instituted."[1] Those propitious times would soon end, however, as a collective mood swing pushed cannabis and its medicinal properties into a limbo of scientific indifference.

And there it stayed for several decades—despite the lonely sounding of a few 'voices crying in the wilderness'[2,3]—until about 10 years ago, when the serendipitous discovery of a brain receptor that binds cannabis-like compounds brought it back into the limelight[4]. The molecular cloning of the first cannabinoid receptor (now called CB_1) was quickly followed by the identification of a second subtype in the immune system (CB_2) and then by the characterization of two endogenous cannabis-like compounds with their attendant pathways of biosynthesis and inactivation[5]. These discoveries eventually led to the chemical syntheses of potent ligands (agonists and antagonists) selective for either receptor subtype, which have provided invaluable clues to help explain how the endogenous cannabinoid system may influence physiological functions as diverse as pain, movement control and blood pressure[5]. At the same time, these tools have rejuvenated Christinson's plea for "a more extensive enquiry" into the medicinal potential of cannabinoids, allowing researchers to test popular notions such as their appetite-stimulating or pain-killing effects, as well as to explore newer avenues of research. In this issue of *Nature Medicine*, a paper by Galve-Roperh *et al.* provides an excellent example of the cannabinoids' therapeutic potential[6].

Galve-Roperh *et al.* report findings that indicate a new cannabinoid-based approach for the treatment of malignant gliomas. Malignant gliomas are a relatively uncommon but uniformly fatal form of brain tumor that can be modeled in rodents by inoculating glioma cells (for example, C6 cells) into the brain parenchyma. The resulting tumor grows very rapidly, leading to the animal's death within 2–3 weeks after the initial cell inoculation. Galve-Roperh *et al.* found that administration of cannabinoid agonists into the tumor by means of an osmotic pump connected to an intracerebral cannula eradicated the tumor in one-third of the inoculated animals, and prolonged the survival of another one-third for up to 6 weeks. In the remaining group of animals, the cancer was insensitive to the cannabinoids and continued its malignant course unhindered. Although incomplete, these findings must be seriously considered, as glial tumors are peculiarly resistant to traditional therapy.



To identify which receptors are involved in the anti-cancer actions of the cannabinoids, Galve-Roperh *et al.* turned to a cell culture system. Based on their earlier observation that C6 glioma cells undergo programmed death (apoptosis) after exposure to a cannabinoid drug, they characterized this effect pharmacologically. Unexpectedly, they discovered that both CB_1 and CB_2 receptors are involved: CB_1 and CB_2 antagonists were able to prevent cannabinoid-induced cell death only if they were added together to the glioma cultures. This finding indicates that each cannabinoid receptor can trigger a full-fledged apoptotic response independently of the other, as long as it is free to interact with an agonist. Does this also occur *in vivo*? An affirmative answer to this question, which Galve-Roperh *et al.* did not address in their study, might be of considerable therapeutic importance. It would indicate that selective CB_2 receptor agonists can arrest the progression of malignant gliomas without exerting the psychotropic and hypotensive effects that accompany the recruitment of central and peripheral CB_1 receptors.

As with many other G_i/G_o-protein-linked receptors, agonist binding of CB_1 and CB_2 receptors causes inhibition of adenyl cyclase activity and stimulation of mitogen-activated protein kinase activity[7]. However, the results reported by Galve-Roperh *et al.* do not support the