Promiscuity in transgenic plants

The ecological risks of genetically modified crops are of greatest concern when there are no inherent barriers to the spread of transgenes through sexual reproduction. This is most likely when transgenes can spread to weedy species through hybridization, or when the crop species itself exists in weedy forms[1]. If the potential recipient of a transgene is a highly selfing species, such as *Arabidopsis thaliana*, this risk is often considered negligible[2]. Here, however, we report results of a field experiment in which transgenic *A. thaliana* showed a dramatically increased ability to donate pollen to nearby wild-type mothers compared with *A. thaliana* mutants expressing the same mutant allele as the transgenic plants.

In the summer of 1996, we planted 144 *A. thaliana* rosettes at random locations in a grid at our field site in central Illinois, at densities reflecting those of nearby *A. thaliana* populations. One-quarter of our plants were homozygous for the dominant and mutant allele of acetolactate synthase, *Csr1-1*; this allele confers resistance to the herbicide chlorsulphuron. Mutants were originally isolated through mutation of the wild-type *A. thaliana* ecotype, Columbia, by ethyl methanesulphonate, and had been backcrossed for six generations[3]. One-quarter of the plants in the field were wild-type Columbia, and the remaining half were Columbia-strain plants transformed to express *Csr1-1* in a pBin vector[4,5]. The latter possessed insertions at a single site, were homozygous for this insertion, and were equally divided between two independently transformed lines.

Plants were grown in the absence of herbicides, and were visited frequently by syrphid flies which consume pollen and nectar. At the season's end, we identified progeny produced through outcrossing by germinating seeds produced by each wild-type Columbia plant on plates containing 100 nM chlorsulphuron. Chlorsulphuron-resistant seedlings were transplanted to the greenhouse, where outcrossing was prevented with pollination bags, and their selfed seeds were collected. We then identified progeny fathered by transgenic *A. thaliana* by germinating selfed seeds on plates containing 50 mg l^{-1} kanamycin (only transgenic fathers were resistant to both chlorsulphuron and kanamycin).

A survey of approximately 100,000 seeds showed that the per-plant outcrossing rate was 0.30% for mutant fathers and 5.98% for transgenic fathers. Transgenic *A. thaliana* were roughly 20 times more likely to outcross than ordinary mutants. We screened a subsample of 281 transgenic progeny with primers specific to each insertion site in order to identify which of the transgenic lines had fathered them. We calculated the outcrossing rates for these two lines as 1.2% and 10.8%. Both transgenic lines showed increased outcrossing relative to the mutant (χ^2 tests, $P<0.003$ in each case), but the two transgenic lines differed in their propensity to outcross ($P<0.001$).

To be certain that seed contamination did not contribute to estimates of outcrossing, we re-plated the seeds from all selfed, resistant plants on chlorsulphuron plates. Contaminants would have been homozygous for resistance whereas progeny resulting from outcrossing would have been heterozygous. Over 99% of our resistant progeny were heterozygous, and only heterozygous individuals were included in our calculations. We also placed known resistant and susceptible seeds on all selection plates to confirm our ability to determine the phenotypes of progeny.

Our results show that wild-type *A. thaliana* are more likely to be fertilized by the pollen of transgenic rather than mutant *A. thaliana* when each expresses the mutant allele *Csr1-1*. Although *A. thaliana* is unlikely to become a pernicious weed, these results show that genetic engineering can substantially increase the probability of transgene escape, even in a species considered to be almost completely selfing.

Our results do not prove that enhanced outcrossing is due to the transgene itself, but rather that a difference in outcrossing between transgenic and mutant plants exists. Because we do not know the underlying genetic mechanism, the generality of our result is unclear at present. Even if enhanced outcrossing is restricted to *Csr1-1*, however, our results are of broad relevance because this transgene has been introduced into dozens of agricultural crops, and is advocated as a selectable marker for plant transformation vectors[6].

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Resistance to the herbicide glyphosate

The News and Views article by Gray and Raybould[1] paints a rosy picture of the future of agricultural crops engineered with resistance to the herbicide glyphosate. It is true that the presence of the glyphosate resistance transgene (the EPSPS gene) in the chloroplast genome of crop plants would eliminate pollen transfer of this gene to weedy relatives. But there have already been large-scale releases of transgenic, glyphosate-resistant ('Roundup Ready') crops, including glyphosate-resistant *Brassica napus* (Canola, varieties Quest and LG3295), which is disturbing as there are many *Brassica* weeds that could potentially hybridize with the engineered *B. napus* (for example, see ref. 2).

Gray and Raybould[1] also say that "[glyphosate] tolerance in weeds does not seem to have evolved, despite extensive use of the herbicide for over 20 years". Although accurate about the extent of glyphosate use, this statement is inaccurate about the occurrence of spontaneous glyphosate resistance. There have been at least two reported cases of glyphosate resistance in *Lolium rigidum* (rigid ryegrass) in Australia. An article by scientists at Monsanto[3] cited by Gray and Raybould mentions one of these, stating that "studies are required to determine the basis of resistance". Monsanto, who produce a commercially available glyphosate ('Roundup'), are collaborating with a group at Charles Sturt University in New South Wales, Australia, to understand the nature of the resistance in this case. More information is available from a transcript of an ABC Radio National discussion (http://www.abc.net.au/rn/talks/bbing/bb970914.htm) and a catalogue of herbicide resistance (http://www.weedscience.com).

It seems foolish to assume that resistance to a herbicide with a single biochemical target *in vivo* would not develop, given time. Yet public concern about the ability of plants to develop resistance to glyphosate has been assuaged. Perhaps this has caused many other 'isolated' incidences of spontaneous glyphosate resistance to go unnoticed. We should all be concerned about the presumed increased use of glyphosate arising from the cultivation of transgenic

glyphosate-resistant crops. It might be time to demote glyphosate from its status as a completely safe, fix-all herbicide.

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Turning off follicular dendritic cells

Follicular dendritic cells (FDCs) are specialized to display intact antigens for recognition by B cells. Here we show that inhibition of lymphotoxin α/β (LTα/β) leads to the disappearance of multiple markers on FDCs within one day, regardless of whether any immune reactions are taking place. Inhibition of the tumour necrosis factor (TNF) pathway was also effective, but only in the absence of a strong antigenic response. These treatments not only prevented trapping of newly formed immune complexes, but also eliminated previously trapped immune complexes on FDCs, rendering the FDC non-functional. Therefore lymphotoxin and/or TNF is required for the continual maintenance of FDC function.

Gene deletion experiments in mice have shown that signalling by both TNF and lymphotoxin is required for FDC development[1–3]. TNF and lymphotoxin make up two separate immunoregulatory pathways[4]. TNF and potentially the soluble LTα form signal through the classic TNF receptor (TNF-R)-mediated pathway. Membrane-bound LTα/β heteromers signal through the LTβ receptor (LTβR)[5], forming a second pathway associated with the development and maintenance of secondary lymphoid organs[1,6].

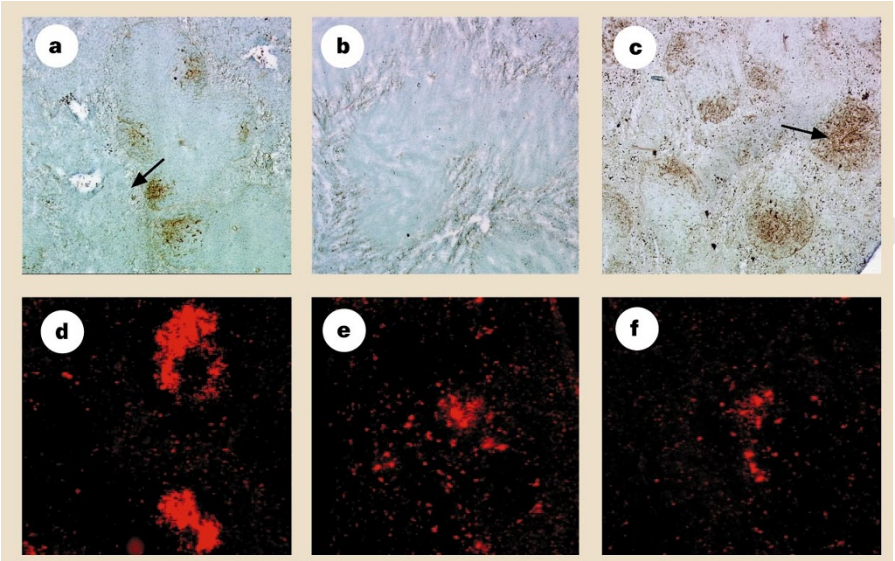

Figure 1 Only the LTα/β pathway is essential for the maintenance of FDC networks following immunization with sheep red blood cells, yet both the TNF and lymphotoxin pathways are required for the maintenance of immune complexes. Mice were treated as previously described[6] with control human immunoglobulin (**a**, **d**), LTβR-Ig (**b**, **e**) or TNFR55-Ig (**c**, **c**). In **a**–**c**, animals were immunized with sheep red blood cells, and frozen sections of the spleen were analysed by immunohistochemistry for the presence of the FDC marker FDC-M1. In **d**–**f**, mice were immunized twice with phycoerythrin, and frozen sections from the spleen collected 8 hours after the last immunization were observed under the fluorescence microscope.

To determine the roles of TNF, LTα/β and CD40L in maintaining mature networks of FDCs, we examined the spleens of normal adult mice treated with LTβR-Ig or anti-LTβ antibody (BB-F6) to block the LTα/β pathway, TNF-R55-Ig to inhibit the TNF/LTα pathway, or anti-CD40L (MR1) to inhibit the CD40L pathway (Table 1). Inhibition of either the TNF or LT pathway in non-immunized mice led within 24 h to the disappearance of the FDC-specific markers FDC-M1, FDC-M2, MAdCAM-1 and CR1. The same observation was made in lymph nodes. The elimination of MAdCAM-1 expression on reticular cells in the marginal zone also tracked with the FDC, whereas the disappearance of markers for marginal-zone macrophages took weeks[6], raising the possibility that these MAdCAM-1-positive cells are FDC precursors. The effect of LTα/β pathway inhibitors was seen with and without immunization with keyhole limpet haemocyanin or sheep red blood cells, and treatment with TNF-R55-Ig had no effect when the animals were immunized (Fig. 1a–c and Table 1).

We investigated the function of FDCs by testing their capacity to trap immune complexes in the spleen[7]. Immune complexes were formed following a secondary immunization with phycoerythrin, and the fluorescent property of the protein allowed immune complexes to be detected in B-cell follicles. These immune complexes are known to colocalize with FDCs[7]. Pretreatment of mice with inhibitors to the LTα/β pathway before immunization with phycoerythrin prevented trapping of immune complexes on FDCs (data not shown). Pretreatment with TNF-R55-Ig did not prevent immune complex trapping, although the pattern of immune complex deposition was altered. Once immune complexes are trapped on FDCs, inhibition of either the LTα/β or TNF pathway now affected the maintenance of these immune complexes (Fig. 1d–f and Table 1). Moreover, because anti-LTβ had the same effect as LTβR-Ig in all cases, involvement of the newly defined LIGHT ligand can be excluded in these functions[8]. Treatment with anti-CD40L antibody had no effect in any of the experiments.

Pretreatment of adult mice with inhibitors of the LTα/β pathway impairs the antibody response to sheep red blood cells[6]. We used phycoerythrin-specific enzyme-linked immunosorbent assays to show that treatment with LTβR-Ig or TNF-R55-Ig did not inhibit the primary or the secondary antibody response to phycoerythrin. Thus,

Table 1 **How TNF and lymphotoxin pathways affect FDC status in the spleen**

Treatment	Control Ig	LTβR-Ig or anti-LTβ	TNFR55-Ig	Anti-CD40L
Detection of FDC in spleen after immunization with				
No antigen	Yes	No	No	Yes
Sheep red blood cells	Yes	No	Yes	Yes
KLH	Yes	No	Yes	Yes
Phycoerythrin*	Yes	No	No	Yes
Deposition of immune complex on FDCs	Yes	No	Yes	Yes
Maintenance of immune complex on FDCs†				
24 h	Normal	Normal	Normal	Normal
48 h	Normal	Reduced	Reduced	Normal
72 h	Normal	Not detected	Not detected	Normal

*Weak antigen.
†Time after treatment of mice previously immunized with phycoerythrin.