the clinic demonstrated that the absolute sizes of their defects had been reduced and, of utmost importance to these individuals, their visual fields had increased. Among the restitution group, 95 percent of subjects showed an average increase in their visual field of 4.9° (post-chiasmatic injury) or 5.8° (optic nerve injury).

In the past, similar improvements in vision associated with training have been reported. One notable series of studies involving patients with damage to visual areas posterior to the thalamus described enlargements of the visual field in 40 of 55 patients following eye-movement training. Another study using similar methods concluded that what at first seemed to be visual field rehabilitation was more likely to be the result of compensatory strategies on the part of subjects, such as biasing their gaze towards their blind regions. Furthermore, these apparent improvements could be eliminated by adopting more rigid experimental controls such as testing with automated perimetry (a standard ophthalmological technique) suggesting that reports of visual restitution should be interpreted with caution.

This study goes well beyond these earlier reports. Improvement is noted not only along the transition zone of the blind region but also at islands of phenomenal vision within the blind area. Furthermore, improvements were noted in patients with optic nerve damage, who have not been studied in previous clinical protocols. In the Kasten study, rigid experimental controls were instigated, such as controlling the direction of gaze during training, maintaining a stable head position, checking and calibrating training profiles during monthly clinic visits, and independently verifying all improvements with automated perimetry.

It is unclear why the recovery was so marked, although one important aspect of the Kasten protocol is that recovery is visual field training dependent. Control subjects who spent equivalent amounts of time using the computer but not performing tasks specific to visual field training, did not experience improvements in vision. Based on converging evidence from both animal and other human studies, the authors propose a minimal residual structure hypothesis, which postulates that specific visual stimulation of as few as 10–15 percent surviving neurons is sufficient for recovery of basic visual functions. Of course, 100 percent survival of neurons of the normal vision pathway is the best possible scenario, but survival of 10–15 percent permits sufficient recovery of rudimentary visual function, making a remarkable difference in the lives of partially blind individuals. One of the most encouraging indications from the Kasten study is that computer training may stimulate the few surviving neurons in islands of vision with awareness. Use of these methods with patients who demonstrate islands of vision without awareness may subsequently result in the development of awareness within these areas.

Visual recovery obtained with a computer-based training program, which can be used in the comfort and convenience of the home, should provide the partially blind with a measure of optimism that some restoration of vision is possible. I look forward to these training opportunities becoming more widely available.


**The art of tolerance**

In a mouse skin transplant model in which donor and recipient mice differ by only one minor histocompatibility antigen, graft tolerance can be induced up to ten days after transplantation, but endures only if there is persistence of migratory antigen (pages 1015-1019).

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viral and transplantation immunology on common ground. Using transgenic techniques, they have produced colonies of animals that express transgenes encoding well characterized viral epitopes. With this technology it has been possible to pool the vast reservoir of information in viral immunology (particularly that acquired for LCMV) with the repository of knowledge in transplantation immunology, and to address controversial questions about transplantation tolerance.

The transgenic donor mice used in the experiments of Ehl et al., reported on page 1015 of this issue, ubiquitously express the gp33 peptide of the LCMV glycoprotein. When cells or tissues from these mice are infused or transplanted into otherwise normal mice of the same strain, the gp33 epitope constitutes a single minor histocompatibility class I-restricted antigen. This results in rejection of the transgenic skin by normal mice of the same strain in 70–120 days. In a subgroup of experiments, the recipient effector system was "pre-armed" by injection of a T-cell receptor transgene that increases the frequency of naive gp33-specific precursor cytotoxic T lymphocytes (CTL). The time to rejection of the gp33-positive skin grafts was reduced to 15 days. These investigators demonstrated that there is an absolutely specific rela-
The tolerance induced by the gp33 expression and its cognate host CTL clone.

In their new experiments, the authors convincingly show that tolerance to the gp33 peptide expressed by the transgenic donor skin grafts can be reliably induced by conditioning the normal recipients in two ways: with three injections of a synthetic gp33 peptide (which is eliminated by the unaltered mouse in about 60 days) or with a single injection of long-lived spleen cells expressing gp33. They assess the relative effectiveness of tolerance inducing protocols through their ability to prolong survival of the gp33-positive skin grafts. Survival results were compared with data on the frequency of gp33-specific CTL precursor cells. Both the short-lived synthetic gp33 and the gp33-expressing spleen cells were tolerogenic, whether given before or up to ten days after skin transplantation. However, the full development and perpetuation of the tolerogenic effect depended on the persistence of gp33 expressed by donor spleen cells (that is, it depends on donor splenocyte chimerism). Tolerance is not sustained by the transgenic skin grafts alone in the animals in which original non-reactivity was induced with synthetic gp33 peptide.

The principles defined in this exceptionally pure model can be readily identified in the more complex setting of clinical transplantation in which two populations of immune competent cells confront each other under an umbrella of immunosuppression. However, in contrast to the simplicity and precision of the Ehl model, four interrelated events, all regulated by antigen migration and localization, must occur in relatively close temporal proximity for organ allografts to be accepted by patients and in most experimental models (see figure). These are: (1) antigen-specific clonal exhaustion/deletion of the recipient immune reaction; (2) reciprocal exhaustion of the allograft immune competent cells (passenger leukocytes) that migrate to host lymphoid organs after transplantation; (3) maintenance of the exhaustion of (1) and (2); and (4) reduction of the organ’s antigenicity by progressive depletion of its passenger leukocytes, making the allograft an object of increasing immune indifference to the host immune system.

The first three of these events are dependent not only on the persistence of antigen, but also on its transport to the milieu of organized lymphoid tissue where growth factors, different cell types, and other factors necessary for efficient immune activation are located. The short-lived synthetic gp33 peptide administered to recipient mice after transplantation in the Ehl experiments has been previously shown by the authors to reach these destinations. However, with bioelimination of the synthetic peptide, tolerance cannot be maintained by the oulying transgenic skin graft, even though the histocompatibility barrier is a weak one (that is, skin graft survival for 70–120 days in unmanipulated recipients). Similarly, the disappearance of microchimerism in an organ recipient presages loss of the oulying allograft due to chronic or acute rejection. In the Ehl model, skin graft rejection was associated with thymus-dependent recovery of precursor CTL.

The tolerance induced by the gp33 expressed in the presumably self-renewing mobile spleen cells is enduring and resistant to tolerance breakdown maneuvers, even though the chimeric cells can no longer be identified by flow cytometry in the blood after 60 days. Terakura et al. have shown in a rodent allograft model that a large proportion of allogeneic leukocytes surviving the early post-transplant period leave the blood and lymphoid organs and find niches in non-lymphoid tissues and organs of the host, such as skin and heart. Such secondary relocation, a phenomenon also seen with widespread non-cytotoxic pathogens, would explain the findings of Ehl et al. and also why blood samples and lymphoid organ biopsies cannot be used to reliably monitor the presence or level of chimerism in organ allograft recipients. Periodic leakage of chimeric cells from the non-lymphoid to the lymphoid compartment after transplantation has been suggested as an explanation for the maintenance of clonal exhaustion. This is comparable to the stable equilibrium between destructive and non-destructive immunity described in an analogous model of autoimmune diabetes mellitus.

Finally, the information provided by the Ehl experiments adds to the evidence that giving adjunct donor leukocyte infusions to organ recipients is an attempt (that can be safely delayed) to augment the normal chain of post-transplantation tolerogenic events. Under the circumstances of conventional organ transplantation, these tolerogenic events depend on the migration of passenger leukocytes from the donor allografts. Although it is disappointing to note that the reported tolerogenic protocols used by the authors could not override the degree of pre-sensitization induced by pretransplant immunization with LCMV, tolerance has been shown by others to be produced in presensitized mice by the large bolus of hepatic passenger leukocytes released into the recipient circulation after liver transplantation.

8. Starzl, T.E. & Zinkernagel, R.M. Antigen localiza-
The journey from folk medicine, to identified receptor, to endogenous ligand, exemplified so elegantly by the opioids, has now been repeated by the cannabinoids, a group of compounds whose members were first isolated from the marijuana plant. The recent development of selective antagonists of the cannabinoid receptors, CB1 and CB2, has permitted a much greater level of certainty in the interpretation of pharmacological data. It is well established that endogenous cannabinoids act primarily via the CB1 receptor to produce significant analgesia in the central nervous system. However, their effects in the periphery were until recently unknown.

Exploiting the newly available selective CB1 and CB2 antagonists, Calignano and colleagues now report in Nature that two endogenous cannabinoids attenuate nociception in the peripheral tissues of the mouse (in which acute and tonic pain is elicited by injection of formalin into the hind paw). Furthermore, these cannabinoids are synergistic in their antinociceptive action.

Since the early sixties, Cannabis sativa (marijuana) has been widely used in Europe and North America as a recreational drug and has been a cause célèbre of the counterculture. However, in other cultural settings and at other times, cannabis has been used as a medicinal herb. In fact, it has been known to Western medicine as an analgesic for over 150 years. Although it was not always a successful treatment, early investigators were impressed by its safety, appetite-promoting properties and the rarity of addiction, particularly when compared with that of opiates.

When the recreational use of cannabis became illegal in the United States with the passage of the Marijuana Tax Act in 1937, its medical use in North America ground to a halt and, consequently, research on its clinical pharmacology was severely curtailed. A few studies showing clinically significant analgesic effects of smoked cannabis or of one of its major purified active ingredients (delta-9-tetrahydrocannabinol) have been published. In contrast to the lack of systematic studies, the illicit but widespread availability of cannabis has led individuals to experiment with the drug and to report its benefits as an appetite stimulant and analgesic. This resulted in recent referenda in California and Arizona that sought to legalize the use of cannabis for the treatment of medical conditions, such as AIDS and cancer, through the widespread medical marijuana has been actively opposed by Federal Government.

A highly specialized and turbocharged research community is trying to build a scientific understanding of cannabinoid analgesia activity, and the arguments who the mechanisms of cannabis pain management. The hypothesis is that because of the mechanism of the analgesic effect of cannabis differs from that of other well-known analgesics (because it acts through cannabinoid receptors, and opioid antagonists do not block its action) it is likely that some patients would benefit from its use.

Broad scientific interest in cannabis has been rekindled by the cloning of the CB1 and CB2 receptors (refs. 4, 5) and has been further spurred by the synthesis of the first selective cannabinoid receptor antagonists, which provided direct evidence for the existence of functional endogenous agonists for the two receptors.

The endogenous cannabinoids anandamide, which acts primarily at the CB1 receptor, and palmitoylethanolamide (PEA), a candidate CB2-like receptor agonist, are synthesized both in the brain and in the peripheral tissues. The CB1 agonists have analgesic activity in the central nervous system and both CB1 and CB2 agonists have peripheral analgesic action. Cannabinoids may also have anti-inflammatory properties.

In their new study, Calignano and colleagues demonstrate that the peripheral analgesic effect of anandamide is reversed by the CB1 antagonist, SR141716A, but not by the CB2 antagonist, SR144528. The analgesic effect of PEA is mechanistically distinct from that of anandamide, and is blocked by the CB2 but not the CB1 antagonist. When administered together into the hind paw (but not into the ventricles of the brain), PEA and anandamide act synergistically to produce analgesia, an effect completely blocked by either the CB1 or the CB2 antagonist.

In some ways the most interesting and potentially clinically relevant result is that, without administration of an agonist, intravenous injection of either the CB1 or CB2 antagonist produces hyperalgesia. This suggests that, under the conditions of the experiment, there is tonic release of endogenous CB1 and CB2 agonists. Consistent with a tonic endocannabinoid peripheral action, the CB1 antagonist is much more potent when injected directly into the paw than when injected intravenously. For technical reasons, the CB2 antagonist could not be tested in this paradigm. Additional support for endogenous cannabinoid release comes from the fact that PEA and anandamide are found in the rat paw at levels sufficient for significant activation of the CB1 and CB2 receptors.

There are some loose ends to this story.