Ever since the demonstration that allografts are rejected through immune reactions of the host, clinical therapies for organ allografts have relied on immune suppression to prevent these destructive events. A growing body of clinical and experimental data suggests that allografts elicit multiple, interactive immune responses. The result is not inevitably graft rejection, and “spontaneous” acceptance of fully allogeneic liver grafts occurs in rodents without immunosuppression. A spectrum of results range from spontaneous acceptance without immunosuppression to rejection with immunosuppression. The “dualistic pathway paradigm” aims to reconcile apparently conflicting observations in liver transplantation and proposes that: (1) immune engagement between the host and the allograft is instrumental in both rejection and acceptance; (2) there exist in all mammalian species congruent interactive pathways of immune activation whereby the fate of the allograft is determined by the quantitative results of these interactions; (3) the dualistic effect of immunosuppressive drugs on pathways of immune activation, conferring the capacity for favorable or unfavorable graft outcome should be investigated in experimental models in which organ allografts are spontaneously accepted. In conclusion the design of clinical strategies based on this research may contribute to protocols resulting in allograft acceptance without chronic immunosuppression. (Liver Transplant 2004;10:1081–1086.)

Compared with other solid-organ transplants, liver allografts have long been considered to be immunologically privileged, as manifest by an absence of hyperacute rejection despite a positive T-cell cross-match, a low incidence of graft loss due to chronic rejection, and the potential of hepatocyte regeneration after tissue injury.1 Despite this special status, two observations made in liver transplantation have been considered as paradoxical and remain poorly understood. First, in contrast with clinical experience, liver allografts in rodents or pigs are often spontaneously accepted across a full major histocompatibility barrier without any immunosuppression although other vascularized organ allografts are acutely rejected2–4; an early heavy cellular infiltrate in the liver graft progressively resolves with no sign of permanent tissue injury. In several fully allogenic donor/recipient strain combinations in the rat, recipients spontaneously accept the liver graft and rapidly become systematically and specifically tolerant for subsequent skin and organ grafts from the donor, but reject third party grafts in normal fashion.5 Second, in human liver allografts, significant lymphocytic portal infiltrates have been observed during the few days or weeks after transplantation, suggesting the occurrence of a potentially destructive acute rejection process; however, it has been shown that such infiltrates do not necessarily indicate an immune injury to the transplant, and accordingly may not require reinforcement of the immunosuppression.6

The aims of this paper are to briefly review the past and current paradigms in liver transplantation immunology, and, on this basis, to derive a working hypothesis, which may allow this set of observations to be reconciled within a conceptual framework that points to new perspectives for preclinical and clinical investigations in this field.

The Paradigms of Liver Transplantation Immunology

The term paradigm refers to an organized group of hypotheses that helps to approach the understanding of natural phenomena: paradigms do not necessarily reflect directly the reality, but rather should be regarded as useful working tools for making progress towards a better understanding of complex issues. Three paradigms have been successively introduced to support the clinical and experimental approaches towards immuno-
suppression in organ transplantation in general, and in liver allografting in particular.

Classical Paradigm of Transplantation Immunology

This linear model (Figure 1) essentially guided the clinical approach towards organ transplantation since the early days some fifty years ago, after the discovery by Medawar that transplant rejection is an immune phenomenon.7 Briefly, according to this concept, any allograft elicits from the host a harmful immunological response leading to acute rejection; this can be prevented by appropriate prophylactic immunosuppression. Within this framework, acute rejection was generally diagnosed using criteria based on graft histology, and was treated by reinforced immunosuppression in order to prevent the ultimate destruction of the transplant secondary to chronic rejection. Accordingly, this paradigm underpinned most of the many clinical trials aimed at the development of efficient immunosuppressive drugs and drug combinations for all vascularized organ grafts.

Systemic and Allograft Microchimerism

As initially published respectively in 1969 and 1992 by Starzl and colleagues, this crucial revolutionary concept was derived from observations that neither the graft tissues nor the recipient soma remain genetically homogeneous following transplantation8,9: several observations in long-term organ recipients suggested that bone marrow-derived passenger leukocytes of donor origin could migrate from the graft and persist widespread but at low levels in the recipient tissues, constituting a state of microchimerism. These events establish a continuing interaction between migrated donor cells and the recipient immune system, a condition viewed as necessary and responsible for the induction of long-term tolerance, and as a consequence, providing an opportunity to withdraw all immunosuppression in certain patients. Unfortunately, this attractive concept has not yet been entirely validated, since the exact nature of interaction(s) between the recipient immune system and migrated donor cells is not fully understood, and no perfect correlation exists between microchimerism and long-term graft tolerance in studies of individual patients.10,11 Thus, it is not yet clear whether microchimerism is a cause or a consequence of tolerance, and, as suggested by Calne, it may be merely an epiphenomenon of tolerance.12 Accordingly, systemic microchimerism is not currently considered as a surrogate marker of immunological tolerance in clinical transplantation.

Immune Activation after Liver Allografting

Liver allografts in pigs and rodents can induce donor-specific tolerance.2–5 However, these transplants show early (1 – 2 weeks post-transplant) biochemical and histological markers of acute rejection, which then progressively and spontaneously recover to normality. Moreover, as depicted in Figure 2 and suggested in the literature, human liver allografts can encounter similar lymphocyte infiltration, which may not necessarily require a reinforcement of the immunosuppressive load.6 From these observations, Calne and colleagues hypothesized that, in the absence of an aggressive T-cell response, some form of engagement of the host immune system towards graft components could be permissive for the development of operational or prope (Latin: “almost”) tolerance.13 Furthermore, it was predicted that accordingly some immunosuppressants might overwhelm such immune engagements, and thereby prevent the development of natural processes leading to an active state of graft acceptance. This concept was further investigated in rodents by Bishop and colleagues, who revisited the rat strain combinations exhibiting spontaneous donor-specific tolerance induced by liver allografting across a full histocompatibility barrier, with the following relevant findings: (1) liver acceptance and tolerance induction is associated with an early and marked activation of the recipient immune response (as assessed by interleukin-2 and
interferon-γ RNA precursors), followed by a subsequent decline manifested as the production of proinflammatory cytokines within the graft, at levels insufficient to prevent recipient T-cell death by neglect14–16; (2) this process of tolerance induction was shown to be abrogated either when passenger leukocytes in the liver graft were inactivated by donor irradiation, or by a short course of methylprednisolone (but not of cyclosporine) immunosuppression administered to the recipient in the early post-transplant period.17–19 Accordingly, these findings suggested that immunosuppressive strategies may impact — through enhancement or abrogation — on the active processes involved in the spontaneous tolerance induction following rat liver allografts.

The Dualistic Pathway Paradigm: A Unifying Hypothesis

Following a previous publication by Bishop and McCaughan in this journal,16 the integration of clinical and experimental findings in liver transplant immunology lead us to derive a working hypothesis, which may be referred to as the dualistic pathway paradigm. This hypothesis was developed in an attempt to reconcile observations of spontaneous induction of tolerance in rodents and of immune activation, which does not always lead to liver allograft destruction in man. As already suggested by other authors, the immune activation process (lymphocyte proliferation and differentiation) occurs normally as an immune response to liver allografting. According to the paradigm proposed, these naturally occurring phenomena may result in two functionally distinct pathways of behavior (Figure 3): (1) a pathway (or a group of pathways) of immune events oriented towards immunologically mediated allograft destruction (rejection pathway) and, (2) a pathway (or a group of pathways) of immune events oriented towards tolerogenesis (tolerogenic pathway). Moreover, the paradigm postulates the existence of qualitatively common mechanisms induced by liver allografting in rodents and in man, which could lead either to rejection or to tolerogenesis depending on the quantitative balance between both (groups of) pathways in the context of species differences (Figure 4). Beside this interspecies variability, this balance may also differ between individuals in outbred populations on a genetically controlled basis. The exact immunological mechanisms underlying the tolerogenic pathway(s) are not yet fully elucidated, but could include the following phenomena20: (1) donor-specific T-cell clonal apoptosis (central of peripheral mechanisms), particularly through the process of activation-induced cell death; (2) anergy
through second signal inhibition); (3) regulation that achieves an active form of tolerance (possibly resulting from specific regulatory cells and/or a Th1/Th2 immune deviation process).

Obviously, highly complex, multi-directional immunological processes have been deliberately reduced to a simplistic model, but like many paradigms, the dualistic pathway hypothesis does more to aid a conceptual understanding than it does reflect reality. However, this paradigm provides a framework that could unify the observations made in rat and human liver transplantation, addressing the issue of immune engagement, with the outcome that new perspectives are opened in both preclinical and clinical researches in the field, including the following issues:

1. As already suggested, the rat model could be considered as a valuable model for the systematic assessment of permissive or inhibitory characteristics of individual and/or combined immunosuppressive drugs (Figure 4). For example, the new and putatively tolerogenic regimen combining pre-

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**Figure 3.** Dualistic pathway paradigm of liver transplant immunology. Liver allografting induces an immune activation process, which may result secondarily in tolerogenic as well as rejection immune events, leading respectively to graft tolerance or destruction. The quantitative balance between both pathways may vary on an interspecies or even an interindividual basis. A tolerogenic immunosuppression would selectively inhibit the rejection pathway, and preserve the mechanisms leading to tolerance induction.

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**Figure 4.** The dualistic pathway paradigm may be viewed as qualitatively similar but inversely balanced in rodent and human liver transplantation. (1) In some strain combinations in rodents, spontaneous tolerance to the donor strain is induced by liver transplantation despite major histocompatibility complex disparity; in this instance however, tolerance may be abrogated by an immunosuppressive intervention (**: donor irradiation or steroids administration to the recipient), which suggests the existence of a latent rejection pathway. (2) In contrast, after human liver transplantation, the rejection pathway is predominant, but it is hypothesized that the tolerogenic component of the immune activation may be enhanced by an appropriate immune intervention (**).
treatment with antithymocyte globulin and subsequent tacrolimus monotherapy recently proposed by the Pittsburgh group could be investigated closely within this framework using rat strain combinations spontaneously tolerant to liver allografts. Similarly, induction therapy with anti-CD25 blockade, rapamycin, and antimetabolites with or without calcineurin inhibitors could also be tested. The results of such investigations should subsequently be assessed in larger outbred animal models in order to trial the concept in preclinical settings. From a clinical perspective, recently published favorable results with steroid-free immunosuppression in liver transplant patients correlate with the deleterious effect of steroids for the development of tolerance in the rat liver model, and thereby could constitute the first clinical application of this concept, and lend support to its validity. Furthermore, it may be that such rationally tested immunosuppressive strategies could allow to guide a safer immunosuppression withdrawal at a defined stage after transplantation.

2. While the precise mechanisms and time course of the immune engagement phenomena urgently need further investigation and characterization, the framework described herein may provide improved conceptual tools to allow differentiation of intragraft lymphocyte infiltrates associated with a destructive process of graft rejection or, in contrast, with a progressive tolerogenic phenomenon.

3. In the clinic, it will be important to monitor different immunological parameters systematically after transplantation in order to identify the immunological phenotype(s) most likely to be associated with tolerance, and to characterize surrogate markers defining individual patients in whom immunosuppression could be tapered or even stopped without prejudice to the healthy function of the graft.

Conclusion

The new vision of transplantation immunology developing recently in the literature may contribute to modify profoundly our classical approach towards organ transplantation as well as the objectives of future preclinical and clinical investigations in the field. In this context, the dual pathway paradigm presented in this essay helps to realize that liver allografting may be spontaneously tolerogenic as such, totally (as in some rat strain combinations), or partially (as in man and domestic swine), and that immunosuppressive regimens should aim at the sparing or even the enhancement of this property. However, this paradigm has the limitation to reduce complex and dynamic processes to simple concepts, deliberately not taking into account the multiple interactions with the ongoing allogenic response, including the putative role of transmitted and intercurrent infections. Nevertheless, within such framework, a better understanding of the immune regulatory mechanisms following experimental and clinical liver transplantation will constitute a crucial step to rationalize the therapeutic approach in this field. Whether such concept may also be valid for extrahepatic allogenic organ transplantation remains to be determined.

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