What Is New?

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The question "what is new?" is a difficult one to answer. Too many people believe that they should deliver so-called news. But, what is "news"? First, that depends on the level of knowledge. The more you already know, the less you will find is new. On the other hand, for the inexperienced young student, almost everything will be new. Second, news depends upon your point of view. Basic researchers, clinical researchers, and practitioners have different ideas about what is interesting. Large quantities of information fill the reservoir for reconfirmation, rediscovery, reevaluation, and/or republication. Third, news depends upon your estimation of its value. For example, is it really new in its own right or does it need to be validated by an independent study? Fourth, news may be a small step in an ongoing study, with an important end result. Finally, news may be new and possibly interesting but not always relevant to the topic in question.

With this in mind, I must note that most of what was presented at the symposium was very worthwhile—and I will restrict myself to a few remarks. I will do so by providing simplified summaries of what is at present known of etiological factors, pathophysiology, prevention, and treatment of chronic rejection (CR), and I will add notes about the most interesting news presented at the symposium. Their order will follow these summaries, not their sequence of presentation. And inevitably, they will be incomplete.

Etiological Factors of Chronic Allograft Dysfunction

Immunological Factors (Alloantigen-Dependent)

1.1 grade of histoincompatibility,

1.2 acute rejection episodes (number, onset, severity, length, reversibility),

1.3 continuing low-grade alloresponse,

1.4 type of immunosuppression (anti-Th1 response or broad) and role of anti-donor antibodies (activated via Th2 response),

1.5 noncompliance of drugs (insufficient immunosuppression), and

1.6 breakdown of a susceptible, regulatory state of immunotolerance.

When giving immune reactions a leading role in CR, it should be remembered that immune reactions are not the "heroes" of self-non-self-discrimination—if they were, clinical medicine would not have to carry the heavy burden of autoimmune diseases. Rather, they represent a lifelong regulation of the consequences of specific recognition of antigens by lymphocytes, such that damage and inflammation is initiated (protective immunity against nonself, in particular pathogenic microorganisms) or avoided (tolerance, in particular, of self-structures). This regulation is a complicated and poorly understood in vivo process, which we want to manipulate because CR is currently the major constraint of successful organ transplantation. Eventually, what we want to see is long-term functional tolerance of nonself.

Along these lines, we heard presentations of important ongoing work that should be mentioned here, though it also belongs to the realm of prevention of CR (see below). The 1st study, as shown by M. Hardy, was about long-lasting tolerance, even of skin grafts, in rats treated with immunogenic major histocompatibility complex (MHC) class I allopeptides, adoptively transferable by primed regulatory T cells. A 2nd important work focused on Il 10-dependent CD45RB^{low}, specifically down-regulating CD4^{+} T cells in mice, as shown by K. Wood. An interesting work consisting of in vitro studies by N. Suciu Foca-Cortesini examined CD8^{+}CD28^{-} MHC class I-restricted human T cells that initiate a suppressor cell cascade via tolerogenic antigen-presenting

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cells (APCs) to tolerizing $CD4^+$ T cells. Subsequent 1st clinical data of R. Cortesini indicated an association with the absence of acute rejection.

Nonimmunological Factors (Immune-Enhancing and/or Per Se Injuring)

2.1 organ allograft quality (brain death, donor age > 50-60 years, hypertension, vascular disease),

2.2 ischemia/reperfusion (injury and recovery from early inflammatory events),

2.3 infection (e.g., cytomegalovirus [CMV], chlamydia pneumoniae),

2.4 toxic side effects of immunosuppressants;

2.5 hypertension of the recipient (cardiovascular disease, CVD),

2.6 hyperlipidemia of the recipient (hypercholesterolemia),

2.7 gender (estrogens vs. androgens in protection against CVD),

2.8 recipient age,

2.9 genetic factors (black recipient, different rodent strains), and

2.10 size of the graft (reduced number of nephrons).

The interplay between the 2 groups of etiological factors may be, at least in part, characterized by an increased weight of nonimmunological risk factors, which may compensate for good control of the alloimmune reactions, and improved immunosuppressants (chemically engineered or bioengineered), which may compensate for nonimmunological risk factors. The relative weight of each risk factor is a matter of ongoing debate.

Regarding such interplay and weight, there was no real "news." However, the lectures of N. L. Tilney gave an excellent overview of the state of the art for the etiological factors of organ allograft quality and ischemia/reperfusion (2.1 and 2.2). The important work of various groups of Tilney's School consists of rat models on organ donor brain death (Gasser et al., Pratschke et al., Laskowski et al., Wilhelm et al.) and on donor hypertension (Pratschke et al., Wilhelm et al.). P. Reinke discussed infection, as an etiological factor for CR (2.3), with a careful analysis of recent work on CMV reactivation in human kidney recipients, indicating infection at extra-graft sites and indirect graft injury. In a lecture by U. Heemann, and in 2 rat model presentations by his group (Heeman et al., Muller et al.), the observation was presented that it is more risky to be a male recipient. This idea was also covered in an retrospective human kidney recipient study in Japan (Inoue et al.). The importance of age as an etiological factor for CR (2.8) was illustrated in rats (Heemann et al.). This study showed a dominant role for recipient age, but it also illustrated that organ transplantation from an old donor into an old recipient has the worst outcome.

Pathophysiology of Chronic Rejection

Nonspecific Initial Events

Transplantation implies injury, which is followed by an inflammatory response. Key factors are

3.1 endothelial cell activation;

3.2 release of cytokines, chemokines, growth factors, and prothrombotic molecules;

3.3 activation of cell-to-cell adhesion mechanisms;

3.4 migration of neutrophils, monocytes/macrophages (including APCs), and T lymphocytes; and

3.5 induction or enhancement of MHC expression on graft cells.

D. Briscoe presented a report on the expression of leukocyte adhesion/transmigration and MHC molecules on activated human vascular endothelial cells and their role in trafficking leukocytes into allografted tissue. Impressively correlating laboratory and clinical work, he studied serial heart graft biopsies and showed, for example, that increased expression of VCAM-1 and MHC II identifies patients at high risk (and that MCP-1 is associated with chronic rejection).

Kinetics of the development of histological lesions are not necessarily dependent on, but occur more rapidly following, an immune response. T cell–deficient rodents do not develop acute rejection and rarely develop chronic rejection. There appears to be a continuum between early reactions and late

outcome. This was also pointed out by N. L. Tilney in his overview and by the above-mentioned data from his group.

An alloimmune response, initiated by the above, develops in organized lymphoid tissue. MHC alloantigen is recognized by $CD4^+$ cells; subsequently, costimulatory pathways activate these cells as well as $CD8^+$ cytotoxic T cells and antibody-producing B lymphocytes. Amplification of the alloimmnue response takes place by pleiotropic and redundant cytokine cascades.

Chronic alloimmune response is not simply a delayed acute response: T cells decline, more self-MHC-restricted T cells (indirect pathway of allorecognition) perpetuate, and antigen-antibody complexes with activated complement accumulate in areas of intimal hyperplasia. Activated macrophages appear to play a pivotal role, in concert with a network of cytokines and growth factors.

A few examples of the factors involved here were discussed during the symposium. Locally produced C3, absent in C3-knockout mice, as a causative factor for allograft nephropathy was discussed by Pratt et al. Koshiba et al. presented data on heparin-binding, epidermal growth factor–like factor expression in coronary graft arteries, as associated with chronic allograft heart vasculopathy in rats. The complex role of interferon-γ in the pathogenesis of graft arteriosclerosis was highlighted in the lecture of G. Tellides.

Regarding the question of the origin of vascular smooth muscle cells, the so far equivocal evidence may now be settled by elegant studies in rats (Hillebrands et al.). These cells in arteriosclerotic lesions in aortic, as well as cardiac, allografts were of recipient origin.

Nevertheless, urgent questions remain to be answered. Which factors play a main role? Which events provide a major path? And, how many alternate pathways exist?

Outcome of Transplantation

Quantity and duration of the trigger, as well as additional factors, determine onset and progress of irreversible chronic lesions. In their later stages, these lesions appear to be largely antigen-independent. Activated and injured endothelial cells appear to be the main inducers of smooth muscle cell proliferation and migration, leading to concentric intimal thickening, resulting in narrowing and ultimate luminal occlusion of arteries and arterioles of the grafted organ. The cascades of humoral factors also activate adventitial and perivascular inflammation and extracellular matrices, leading to thickening of basal membranes and interstitial fibrosis.

Organ-related aspects in humans, as seen from a clinical perspective, were discussed relatively broadly. P. Neuhaus and B. Portmann reported that the liver appears to be a peculiar graft with remarkable regenerative potential and a much better long-term survival rate (less CR) than kidney, in spite of transplant antigen expression on vascular endothelial and bile duct cells, corresponding histological lesions, and partly, a seemingly de novo "autoimmune" form of hepatitis of chronic rejection. U. T. Hopt discussed chronic pancreas dysfunction as a distinct form of CR, differential dependency of exocrine (high) versus endocrine tissue (low) on ischemia/reperfusion, loss of highly immunogenic exocrine tissue from CR reactions while a sufficient number of islet cells is still insulin producing, and so on. D. Farmer discussed intestinal allografts and their high immunogenicity requiring high-dose, multi-immunosuppressant protocols, still yielding 70% to 100% acute rejection episodes and the highest CMV infection rate (40%-50%) among all organ allografts. Clear CR data are still lacking. J. Klempnauer presented the ever-increasing Hannover renal transplant data, indicating a clear impact of acute rejection on CR, particularly when occurring > 6 months after transplantation. On the other hand, he found a 50% patient death rate with functioning grafts 10 years after transplantation.

Several papers dealt with mechanisms of CR versus long-term function of various organs. Kidney-related aspects were discussed by L. Paul, and pancreatic islets were discussed by K. Ulrichs, who also reported on long-term-functioning encapsulated discordant xenografts. Intestines in various rat models were discussed by W. Timmermann and Meyer et al., whereas liver, again in a rat model, was discussed by Gassel et al.

Prevention and Treatment of Chronic Rejection

Unfortunately, the terms *prevention* and *treatment* are often used indiscriminately, probably because

both are modalities of manipulation—though principally different ones. As far as they are studied in models, some serious problems regarding the generalization of news are often neglected. Mainly just one rodent inbred strain combination is studied; however, humans are outbred, and this provides us with data based on $n = 1$ in genetic terms. Since there may be species differences regarding the relative role of the various factors and mechanisms, the much more expensive (and outbred) primates would be the much more relevant preclinical models.

At this symposium, no primate studies were reported, but R. E. Morris briefly discussed this point with regard to the problems of immunotolerance induction models.

Prevention may be achieved by

4.1 diminution of nonimmunological risk factors,

4.2 attenuation of nonimmunological dysfunction reactions, and/or

4.3 induction of long-term immunotolerance (achieving at least "almost tolerance").

These approaches are not mutually exclusive, and the complex process of CR will require multiapproaches.

Diminution of nonimmunological risk factors was discussed to some extent at this symposium. There is a wide variety of approaches to reduce, or even avoid, nonimmunological etiological factors, as mentioned above.

Attenuation on nonimmunological dysfunction reactions concerns a broad and still poorly understood field, where both preventive and therapeutic approaches could be applied. For prevention, a single intraluminal oligodeoxynucleotide delivery, antisense or decoy (Suzuki et al.), was effective by down-regulating cardiac graft arterial neointima formation. CD31-expression on graft endothelial cells may play a protective role for the development of graft arteriosclerosis, possibly by preventing excessive macrophage infiltration, as shown in CD31-knockout mice (Ensminger et al.).

Recent immunotolerance approaches have been reported above, but it should be added that this important topic reappeared several times during the symposium. A. Thomson discussed, in particular, the key role of (immature) dendritic cells, since DC may promote immunity as well as tolerance, and presented 5 different conceptual tolerance models providing both answers and more questions. J. Madsen pointed out that CR may take place in models with primarily well-established immunotolerance, presuming that (besides the role of other factors) a kind of immunogenic epitope spreading may require continuous tolerance tailoring to maintain it. The role of "passenger leukocytes" for spontaneous rat liver tolerance was newly delineated (Dresske et al.). Long-term acceptance of lung allografts was shown in 3 out of 6 minipigs, using a clinically more feasible protocol of sublethal irradiation and temporary immunosuppressant application without donor bone marrow infusion for hematopoietic chimerism (Strüber et al.). Furthermore, the possible future usefulness of preimplantation-derived, embryonic-like stem cells for the induction of stabile tolerance by the establishment of lasting cell chimerism was discussed by F. Fändrich.

Treatment may be achieved by attenuation of nonimmunological chronic dysfunction reactions, immunosuppression, and/or psychological concern about compliance, among others. All of these fields are broad. For example, regarding immunosuppressants (chemically engineered or bioengineered), we may need broader modes of action (e.g., broader than anti-Th1), reaction phase-adaptation (e.g., early versus late rejection), less long-term side effects (e.g., new combinations), and ultimately their use as means for tolerance induction. These fields have been poorly covered at this symposium, and psychology (understandably?) not at all.

Treatments of nonimmunological chronic dysfunction reactions were discussed, unfortunately only privately, by P. Häyry, who stated that anti-smooth muscle cell drugs were required for treatment rather than anti-T cell drugs. Soluble factor antagonists may also become helpful. It was shown that inhibition of Il-4 activity significantly reduced eosinophil infiltration and the level of intimal proliferation in mouse aorta grafts (Ensminger et al.). Therapeutic agents against both nonimmunological and immune-mediated injury were discussed by R. E. Morris, who concentrated on the clinically established immunosuppressant rapamycine (sirolimus) and, to some extent, on leflunomide,

pointing out its efficacy against smooth muscle cell proliferation. Finally, T. Hünig reported on his extensive basic studies (not especially related to CR) on immune response modulation with so-called CD28 superagonists. These may, in contrast to other CD28 monoclonal antibodies, turn off the known CD28-mediated secondary T cell activation signals and up-regulate, rather than down-regulate, lymph node reactions and initiate TH2-type anti-inflammatory responses.

Summary

- Old news is reconfirmed. In particular, immunotolerance is of central interest.
- Among the new "news": Of the nonimmunological etiological factors, it is the transplant organ quality that attracts particular, recent interest.
- Regarding the factors in chronic allograft response: Involvement of many factors is reported, but their relative importance remains to be defined.
- Many data from experimental animals and clinical experience (which means data from different mammals) appear to converge. They may continue to do so even further as primate models become more involved. This convergence becomes particularly apparent when experienced transplant clinicians and experimental researchers meet and discuss—a special merit of this symposium.

Altogether, and as final news, this was an excellent meeting, well sponsored, most efficiently organized and guided, and provided with a beautiful social program.