Transplantation Reviews 33 (2019) 173–181

Contents lists available at ScienceDirect

Transplantation Reviews

journal homepage: www.elsevier.com/locate/trre

Review article

Chronic rejection after intestinal transplantation: A systematic review of experimental models

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ARTICLE INFO

Keywords:
Animal model
Chronic rejection, intestinal failure
Short bowel syndrome
Tolerance

ABSTRACT

Intestinal transplantation (ITX) constitutes a salvage treatment for irreversible intestinal failure and failure of parenteral nutrition. Chronic rejection (CR) remains the key obstacle for long-term intestinal graft survival but the pathomechanisms are incompletely understood. This study systematically reviews experimental models addressing CR after ITX in order to summarize current knowledge on CR pathogenesis and identify promising experimental strategies. A systematic literature search was conducted in line with the PRISMA guidelines, and 68 out of 677 articles qualified for the final analysis. The average methodological quality of the studies was suboptimal with 7 out of 11 points as assessed by a modified Oxford Centre for Evidence-Based Medicine score. Histology of the chronically rejected graft was almost universally integrated as outcome parameter but we found significant heterogeneity in utilized transplant techniques, organ preservation, immunosuppression and time points of CR-assessment. Several studies identified cellular and humoral immunologic mechanisms in chronic intestinal rejection. Yet, neither preventive nor therapeutic strategies against CR have been successfully introduced into human intestinal transplantation highlighting the persistent need for optimized experimental models. In this review, we aim to improve the translational value of forthcoming investigations on CR by discussing the experimental status quo and potential innovative approaches.

Contents

Abbreviations: CR, chronic rejection; ITX, intestinal transplantation; POD, postoperative day; TPN, total parenteral nutrition.

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https://doi.org/10.1016/j.trre.2019.04.001
0955-470X/

1. Introduction

Although short term- and acute rejection (ACR)-related mortality after ITX has declined in specialized intestinal rehabilitation and transplantation centers over the last decades, the success of intestinal transplantation to treat irreversible intestinal failure is hindered by mediocre long term outcomes and is failing to replace home total parenteral nutrition (TPN) in many countries. The main cause of long-term graft failure after human isolated intestinal transplantation is what has become known as “chronic intestinal graft rejection” or “chronic allograft enteropathy” [1]. This notion of CR describes a progressive and irreversible disease resistant to anti-rejection treatment ultimately requiring allograft enterectomy and/or re-transplantation, the relative incidence of chronic allograft enteropathy in human ITX has been identified in the range of 7–17% as layed out in a recent review by Lauro et al. [2]. Graft fibrosis, enteric neurodegeneration and obliterative arteriopathy are the hallmark features of end-stage intestinal CR and ultimately result in splanchnic hypoperfusion and intestinal dysmotility with functional graft failure [3]. Despite the existence of the intestinal transplant registry database which covers almost all ITX procedures performed worldwide since 1985, the knowledge on the pathogenesis of human CR is still incomplete [4]. While the functional and descriptive attributes of intestinal CR have been well described, its underlying immunological and physiologic mechanisms remain elusive. It is therefore necessary to analyze CR pathogenesis and develop novel strategies for CR prevention and treatment in experimental models with the aim of future translation into clinical practice. Many experimental models of CR after ITX have been published and some may be better suited than others to address the multifactorial complexity of CR after ITX. We hence identified and compared the available literature with the goal to identify the most accurate and promising experimental approaches to CR after ITX at present. Furthermore, we aimed to identify optimal experimental setup, methods, time points and readout parameters to evaluate the onset and severity of CR. Summarizing the existing body of experimental data, we further studied which pathophysiological mechanisms have been described to contribute to CR and which different experimental therapeutic approaches were attempted until today.

2. Material and methods

The systematic review was conducted using the PRISMA guidelines [5]. A systematic search of the MEDLINE/PubMed, Excerpta Medica dataBASE (EMBASE) and Cochrane Central Register of Controlled Trials (CENTRAL) databases was performed covering a 58-year timespan from 1960 to 2018 (Fig. 1) [6–8]. The search terms “small bowel”, “intestine”, and “multivisceral” were combined with “transplantation”, “organ transplant”, “chronic rejection”, “long term survival”, and “allograft injury”. The literature search was restricted to animal studies. Only original full-text papers assessing histologically proven chronic rejection in animal models were considered. Chronic rejection was defined as graft rejection that occurred at least 60 days after intestinal transplantation. Of note, earlier changes may be mostly attributable to acute rejection and/or ischemia reperfusion injury or technical problems at time of transplant and, typically, distinctive features of CR do not develop before the mentioned timeframe of 60 days postoperatively. As further inclusion criteria, all studies had to report the use of allogenic combinations for transplantation. Publications that did not meet the above defined inclusion criteria, studies lacking a control group and review articles were excluded. All eligible studies were classified according to the level of evidence by using an adapted Oxford Centre for Evidence-Based Medicine score (Table 1) [9]. The methodological quality was independently assessed by two reviewers (KK and MVW) and discrepancies were resolved by a third reviewer (NS). One point each was given for any sufficiently covered item, and consequently a maximum of 11 points could be reached. The final score was then calculated as a percentage of fulfilled items.

3. Results

3.1. Methodological quality of reported studies

68 studies out of 677 screened publications qualified for this review (Fig. 1). The mean methodological quality score was suboptimal with 7 out of 11 items or 63%. There were several quality items which were attained in less than 20% of the included studies, these were “randomization of animals across groups”, “blinded outcome assessment”, and “number of animals excluded from the analysis given”.

3.2. Technical characteristics of published CR models after experimental ITX

The majority of studies on chronic intestinal rejection was performed after 1983 when clinical intestinal transplantation resurged with the introduction of the novel immunosuppressants Cyclosporin A and Tacrolimus. We categorized the identified studies in terms of the respective species, strain combinations for the rat models, transplantation and preservation technique as well as the overall study goal.

The main study goals as stated in the abstracts and full texts were identified as a) establishment of an intestinal CR model ($n = 13$), b) evaluation of intestinal CR pathogenesis ($n = 26$), c) testing therapeutic strategies against intestinal CR ($n = 27$) and d) technical considerations in intestinal CR ($n = 2$). After initial studies in canine models in the early era [10–19], rat ITX models were most commonly employed since the 90ies [20]. Murine and pig models for ITX were utilized only in a minority of the publications (Fig. 2) [21–26]. To further specify the rat models, we analyzed the used strain combinations. Up to the year 2006, various strain combinations were utilized in an attempt to create a representative model for human chronic intestinal graft rejection but in the more recent studies dated after 2007 the research focused mainly on F344 (Fischer) rats as donors and Lewis rats as recipients (Fig. 2) [27–33]. Orthotopic isolated ITX was found to be the most frequently performed experimental transplant technique with 50 studies, while heterotopic isolated ITX was reported in 13 studies (Fig. 2B). Only 5 studies reported experimental combined organ transplantation with liver-intestine transplantation in 4 studies and multivisceral transplantation in a single study (all were rat studies). Despite the widespread use of organ preservation solutions such as UW/HTK as the gold standard of organ preservation in human intestinal transplantation, normal saline or lactated Ringer's solution were routinely deployed in experimental organ preservation (Fig. 2B).

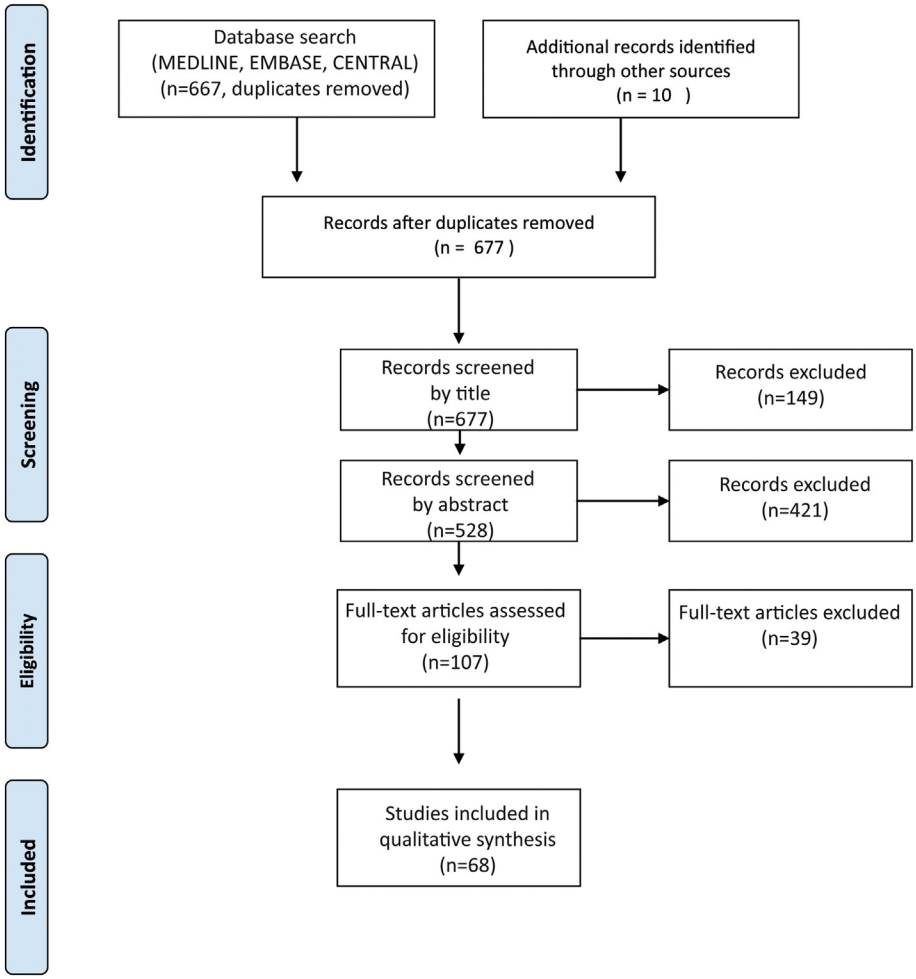

Fig. 1. Literature search results and study inclusion flow chart adapted from the PRISMA statement.

3.3. Immunosuppression in CR models after experimental ITX

The immunosuppressive regimen is a crucial factor in avoiding acute rejection while allowing for sufficient host immunocompetence for the development of CR, as recently demonstrated at our institution (8). Several different immunosuppressive regimens were found, most of which relied on the use of calcineurin inhibitors. The most frequently administered immunosuppressive strategies were compiled from immunosuppressive regimens in index studies in Table 2. In total, 38 studies reported the use of Cyclosporin A and 25 studies reported the use of Tacrolimus. The remaining 5 studies evaluated CR after experimental ITX in an immunosuppressive-free model. While Cyclosporine A and Tacrolimus were equally used from 1990 to 2006, immunosuppression with Tacrolimus clearly dominated the more recent studies. Cyclosporin A was combined with Prednisolone in one study and with both Azathioprine and Prednisolone in a total of four studies. Tacrolimus was also combined with Prednisone in one study and with Rapamycin in another work. An overview of the different CNI-based immunosuppressive regimens over time is given in Fig. 3 for all studies. For a subgroup of index studies, the detailed dosing regimens are listed along with the outcomes and the methodological quality score in Table 2.

Table 1
Methodological quality score assessment.

Item	Description	Points
1	Details of the animals used, including species, strain, sex, and weight described?	1
2	Details of housing and husbandry described?	1
3	Animals randomized across groups?	1
4	Group characteristics clearly described?	1
5	Correct control group used?	1
6	Blinded outcome assessment?	1
7	Transplantation protocols, including TX technique, graft ischemia time, preservation solutions clearly described?	1
8	Time and methods of outcome measurement clearly described?	1
9	Number of animals in each experiment / each group clear?	1
10	Number of animals excluded from analysis clear?	1
11	Details of the statistical methods used for each analysis described?	1
	Total points: max	11

3.4. Time points of CR analysis in experimental studies

The optimal time point to assess end-stage CR in an experimental model of intestinal transplantation would ideally show clear signs of CR in a majority of animals without the punishment of high mortality due to malnourishment and progressing intestinal graft failure while minimizing laboratory animal discomfort. Therefore, it is a tradeoff between allowing for enough time for CR to develop and analyzing the transplanted animals and grafts “on time”. Certainly, the type and dose of immunosuppression is interrelated with the time of CR development. For CR assessment, an interval of 60 days and longer was chosen in 9 studies while an interval of 90 days or longer was reported in 29 studies. 18 studies analyzed graft rejection beyond 120 days and in 6 studies a time point of 200 days or over 365 days was reported, respectively.

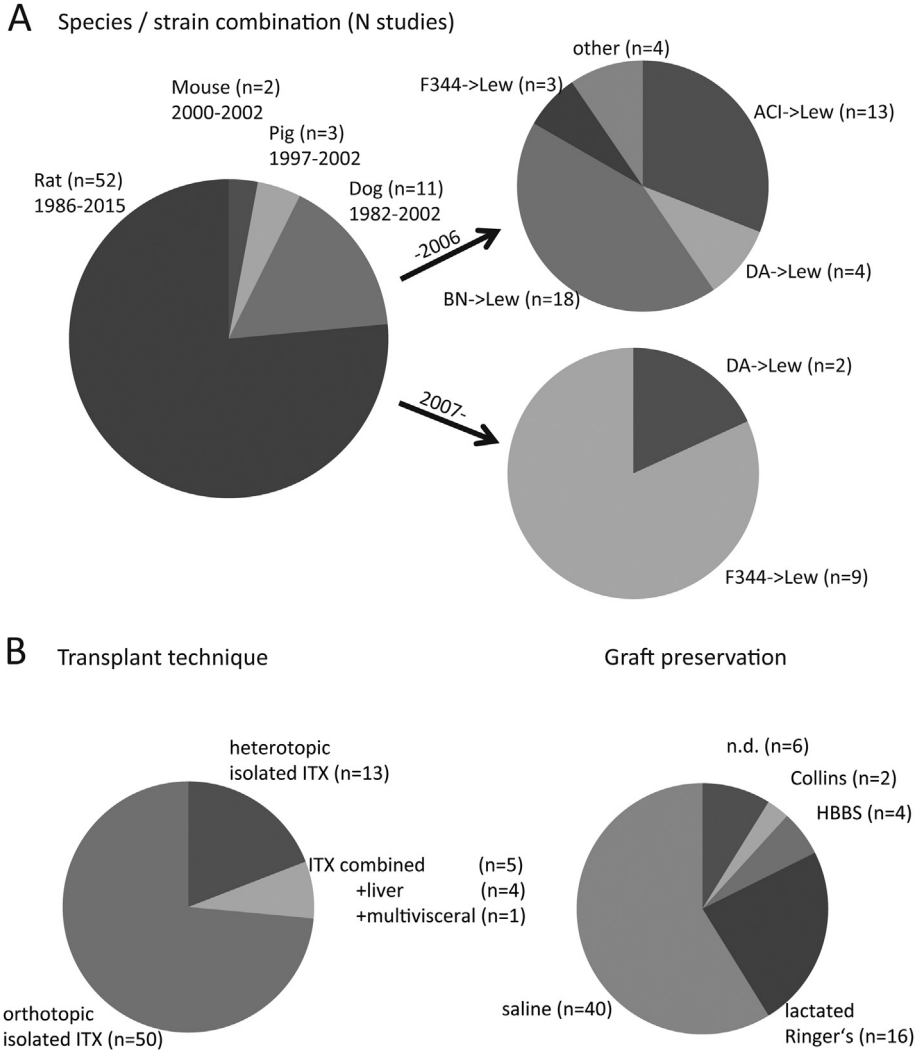

Fig. 2. A/B. Technical characteristics of published CR models after experimental ITX. 2A:Various animal species were historically used for experimental intestinal transplantation. In the last decade, scientists focused on rat models to mimic CR in human intestinal transplantation, with the most frequently used strain combination being Fisher rats (F344) as donors and Lewis rats (Lew) as recipient animals. 2B: Isolated orthotopic ITX was the most commonly employed transplant technique in experimental studies of chronic intestinal rejection. Many historic studies did not use specifically designed organ preservation solutions (B).

Table 2
Immunosuppressive therapy for prevention and rescue treatment of ACR in index studies on experimental models of CR after ITX.

Author	Species/strain	Therapy, dosage	Outcomes	Qualityscore (%)
Li, 2013	Rat, F344 to LEW	Cyclosporine 5 mg/kg/d, day 0–13 FK506 0.3/0.5/1 mg/kg/d day 0–13, 20,27	FK 1 mg/kg/d survived up to POD 180 with classic signs of CR	64
Timmermann, 2000	Rat, BN to LEW	FK506, 2.0 mg/kg/d, day 0–9 (long term)	survived without any signs of CR (>POD 200)	60
Bruin, 2000	Rat, DA to AS	Cyclosporine, 5 mg/kg/d, (long term), 3times a week until 50POD	delayed the development of CR (POD 100)	55
Lee, 1995	Rat, ACI to LEW	FK506, 1.0 mg/kg/d, day 90–120 (rescue)	halted smooth muscle proliferation, improved contractile activity and neuromuscular transmission (POD 120)	64
Lee, 1993	Rat, BN to LEW	FK506, 2.0 mg/kg/d, day 14,16 and 18 (rescue)	prolonged survival by treating acute rejection, but all exhibited features of C.R. (POD 200)	82
Langrehr, 1993	Rat, ACI to LEW	FK506, 2.0 mg/kg/d, 5 days, (rescue),	weight loss(50 g) occurred	70
Langrehr, 1993	Rat, ACI to LEW	Cyclosporine, 15 mg/kg/d, 5 days, (rescue),	did not improve survival (>POD 80), weight loss(50 g) occurred	70
Langrehr, 1993	Rat, ACI to LEW	Cyclosporine, 15 mg/kg/d, 4 weeks, (rescue)	Improved survival temporary without histological alteration (>POD 100), weight loss(50 g) occurred	70

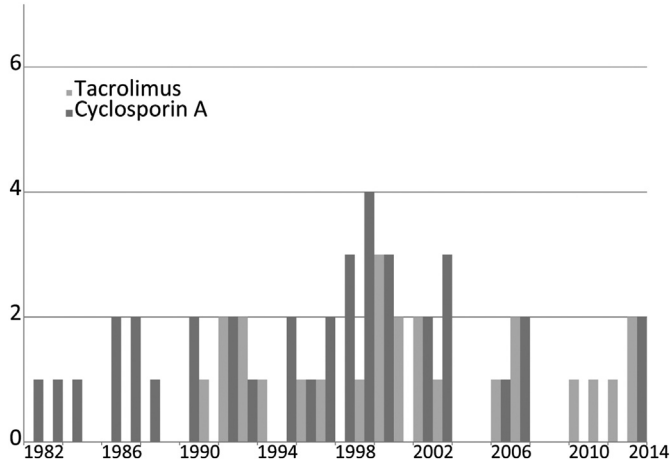

Fig. 3. Evolution of the different CNI-based immunosuppressive regimens in CR models after experimental ITX. Most studies used a CNI-based regimen, with FK 506 (Tacrolimus) being the most popular drug choice in the recent studies.

3.5. Methods of CR assessment and outcome in experimental ITX

3.5.1. Clinical signs of CR

26 studies out of 69 studies (38%) described clinical signs of chronic intestinal rejection as part of the outcome assessment. These signs included weight loss (19 studies), diarrhea (14 studies), palpable abdominal mass (5 studies), alterations in stoma color (1 study), hair loss (1 study) or even animal death in 5 studies.

3.5.2. Macroscopic graft appearance

In 20 studies (29%), macroscopic changes in graft appearance were reported on organ harvest at the predetermined study end or at time of animal death. Intestinal wall thickening of the graft was mentioned in 14 studies. Macroscopic thickening of the graft mesentery and mesenteric lymph nodes were reported in 12 and 9 studies, respectively. Less common features were intensified adhesion formation (6 studies), scarring/depletion of Peyer's patches and development of ascites (3 studies each) as well as splenomegaly and marked bowel dilation (2 studies each).

3.5.3. Graft histology

Expectedly, all except 2 studies included a histologic evaluation of the chronically rejected intestinal grafts. Obliterative vasculopathy (42 studies) was the most commonly reported feature, followed by mesenteric fibrosis or inflammation (23 studies), tunica muscularis fibrosis or inflammation (20 studies), villous blunting (16 studies), and mesenteric lymph node fibrosis (13 studies). Epithelial apoptotic bodies were counted in 7 studies and 6 studies found mucosal ulcerations. We recommend the inclusion of more histologic features (for example goblet cell depletion, scarring of Peyer's patches and more), possibly implemented in a scoring system to be used by two independent reviewers as outlined in the discussion section.

3.5.4. Graft function

Graft nutritive and contractile function was evaluated only in 17 of the included 69 studies. In 12 studies, an enteral absorption test was performed: maltose absorption was done in 6 studies, a D-xylose absorption test was carried out in 3 studies and fat absorption, glutamine absorption and evaluation of the lactulose/mannitol absoption ratio were performed in 1 study each. 7 studies measured myoelectrical activity of the intestinal graft muscularis and 6 studies assessed graft jejunal contractility.

3.5.5. Cell population analysis and molecular observations

A great variety of cellular and molecular analysis was performed to assess CR. A synopsis of all evaluated factors is given in Table 3A. Of note, even though B-cells were studied, we found no animal studies that evaluated the significance of antibody-mediated rejection or the presence of preformed or de-novo donor-specific antibodies in chronic intestinal rejection. We then further correlated the biomarkers with the findings of the included studies and classified them into two main categories: factors observed in association with more severe CR as opposed to factors associated with ameliorated CR (Fig. 3B). The experimental key studies on CR amelioration were additionally categorized into (i) attempts to induce chimerism, (ii) studies on the "liver protective effect" and (iii) others (see Supplement 1) (Table 3B).

4. Discussion

CR currently constitutes the highest and still unresolved hurdle for successful human intestinal transplantation. The mechanisms of chronic graft dysfunction and chronic rejection remain incompletely understood: Firstly, because human intestinal transplantation is less common than transplantation of other solid organs. Secondly, experimental data on CR is limited and difficult to compare. Lastly, no consensus exists on either the definition of CR after experimental ITX, the ideal choice of the technically challenging experimental model or the optimal characterization of the chronically rejecting intestinal graft.

This systematic review of the literature summarizes the experimental body of work published on CR after ITX. We identified 68 studies with an acceptable quality according to predefined criteria and collate the results in this article. From this work, conclusions can be drawn about (i) the suitability of the available experimental models, (ii) elucidation of known pathomechanisms of CR and (iii) pathognomonic attributes and biomarkers of CR. Equally important, we highlight missing pieces in our understanding of chronic graft failure and rejection after intestinal transplantation to possibly stimulate necessary future research.

Table 3A
Cellular and molecular analysis in CR after experimental ITX.

Cytokines	IL-2 receptor (1), IL-4 (1), IL-6 (1), IL-10 (1)	
	TNFa (1), bFGF (2), EGF (1), TGF-b (1), IFN-G (1)	
Other	RAGE, HMGB1 (1), Shh, VEGF (1), iNOS (1), Vimentin(1), Factor VIII(1), CD3 (1), aSMA(1)	
	IgA(1), Enteric nervous system: NADPH, RT97 (2)	
Antibodies	No studies	No donor specific antibody studies
Cellular markers	General	Chimerism studies (anti-donor)
	T-cells (3)	T cells (5)
	$CD4^+$ (5)	$CD4^+$ (3)
	$CD8^+$ (7)	$CD8^+$ (1)
		CD11b(1)
	B-cells (4)	B-cells (2)
	NK-cells (4)	NK-cells (4)
		$CD3^+$ (1)
	$ED1^+$ (3)	$ED1^+$ (2)
	$ED2^+$ (2)	$ED2^+$ (1)
	MHC class II (4)	MHC class I (4)
	Monocytes (3)	
	Mast cells (1)	
	$ED3^+$ (1)	
	iCAM (1)	
	$CD134^+$ (1)	
	Inflammatory cellular infiltrate (1)	
	Recipient-derived cellular infiltrate (1)	

Table 3B
Protective and aggravating factors of CR in experimental ITX.

Associated with less CR (protective effect)	Associated with more CR (aggravating effect)
Anti-CD154+ antibody + donor-specific Splenocyte preconditioning	Heterotopic graft position
Combined costimulation blockade + busulfan + bone marrow infusion	Ischemia-reperfusion injury
Bone marrow transfusion (undepleted)	Graft irradiation
Bone marrow transfusion (T-cell depleted)	Episodes of acute rejection
Treatment with antilymphocyte serum	Cytomegalovirus infection
Co-transplantation of the liver	Impaired reabsorption of bile acids
Lymphatic reconstruction	Mucosal B-cell depletion
Treatment with poly-unsaturated fatty acids	Mucosal NK cells
Treatment with anti-Shh antibodies	Mast cells (IL-4 expression, histamine release→ graft fibrosis)
Short course FK506 treatment	Endothelial vimentin
Long term FK506 treatment	Endothelial factor VIII expression
Long term CyA treatment	Inflammatory cellular infiltrate in lamina propria
Preserved tight junctions	Enteric neuron damage
iNOS expressing monocytes/macrophages	TGF-b, bFGF (fibrosis)
Increased cholesterol synthesis	
EGF	Rage, HMGB-1
TGF-β (immunosuppression)	Shh
	VEGF
	aSMA
	IL6, TNFα, IFN-γ, IL-10 (?)
	CD3+, CD4+, CD8+, CD68+
	ICAM-1
	MHC class II

4.1. Which species and technique can be used to assess CR in an experimental model?

The technique of experimental intestinal transplantation has been historically developed in large animal models including dogs and pigs, but the rat has emerged as the species in which the procedure can be performed in a standardized manner with good survival and an acceptable tradeoff between cost, spatial requirements and technical feasibility. As far as rat strain combinations are concerned, the F344 donor / Lewis recipient model is the most widely used combination to study chronic intestinal rejection in the more recent literature [27–33]. This is best explained by the fact that the F344 donor / Lewis recipient model has a lower immunogenicity and is therefore thought to be more suitable for studies on CR in contrast to the ACR-prone BN donor / Lewis recipient model. However, the main disadvantage of rat models at present is the paucity of transgenic tools for in-depth immunologic analysis in contrast to mouse models. So far, due to technical limitations, no large series of ITX or MVTX in mice has been published.

Generally, it should be kept in mind that experimental ITX is technically demanding. The experimental surgeon undergoes a considerable learning curve before technical variability is kept to a minimum which is a prerequisite for valid results. A controversial point is raised by the choice of orthotopic versus heterotopic ITX: For several reasons and backed by our own experience, we favor orthotopic intestinal transplantation with portocaval venous drainage and restoration of enteral continuity as gold-standard technique of experimental intestinal transplantation, because it best mimicks the human ITX scenario [34]. The main disadvantage of heterotopically transplanted intestinal grafts is the disruption of the normal gastrointestinal transit resulting in mucosal atrophy and significant changes in the microbiome with profound effects on inflammatory processes in the intestine and thus possibly influencing immune processes in the graft [35,36]. Heterotopic ITX holds its' own technical challenges and shortcomings with regard to animal survival or morbidity due to increased stoma complications that have been described [37]. Thus orthotopic ITX remains the preferred technique in the author's opinion, a view which is also reflected in the literature where we identified 50 studies with orthotopic ITX and only 13 studies with heterotopic ITX. However, if co-transplantation with other organs is the goal to study mechanisms like GVHD or the immunoprotective effect of combined liver/intestinal or multivisceral allografts, choosing a heterotopic model might be a feasible option.

4.2. How can CR be characterized in experimental models?

Outcome analysis of ITX and evaluation for CR is currently based on three main parameters: clinical observation of the transplanted animal, macroscopic intestinal graft morphology and intestinal graft histology. Of all clinical parameters, weight loss due to nutrient malabsorption is most frequently reported as a valid element of chronic intestinal rejection. Great care must be taken to comply with ethical animal care guidelines, and fixed endpoints and criteria for discontinuation of the experiment in case of persistent diarrhea or obvious animal deterioration should be defined beforehand.

Macroscopic evaluation of the intestinal graft should be performed in a uniform manner after enterectomy. Mesenterial fibrosis or scarring and mesenteric lymph node atrophy are well established features of CR in the intestinal allograft. A standardized score sheet or high quality photographs can be helpful to assess these morphologic alterations and distinguish between grafts already displaying CR versus still healthy grafts as it is well-known that significant interindividual variation occurs between animals in experimental models of intestinal CR. Intestinal graft histology remains the gold standard to describe the severity of CR. As clearly described in a review by Ruiz, the knowledge on pathologic features of human chronic allograft enteropathy largely stems from explanted grafts [38].

Among other features, microvilli fibrosis and a marked arteriolopathy with perivascular fibrosis and intimal hyperplasia especially in the submucosal and mesenteric arterioles is pathognomonic for intestinal CR and assumed to result from extravasation and subendothelial accumulation of lymphocytes, monocytes and macrophages into the vessel wall [39]. One of the few sequential rodent studies showed mesenterial inflammation after 4 weeks, which consecutively progressed to severe graft fibrosis [40]. Loss of goblet cells and a depletion of Peyer's patches has been also described and the option to follow the same individual through the course of CR may provide valuable insight [41,42]. The intestinal samples should be graded for CR, ideally in a blinded fashion by two independent reviewers, using standardized grading systems for CR analogous to the Wu score in acute rejection but in an adapted fashion for evaluation of CR as described in works by Ma et al. and Schraut to facilitate reproducible histologic assessment [29,43,44]. For immunologic analyses, T, B and NK-cells can be isolated from the graft lamina propria or from the mesenteric lymph nodes (Rumbo, M: personal communication). It seems therefore important to sample peyer's patches and mesenterial tissue. In our experience, isolation of lymphoid and myeloid cells from the mesenteric lymph nodes (mLN) is technically easier than such sampling from the intestinal wall compartments. Importantly, the mLN may represent the “first frontier of recipient/donor leucocyte interaction”, and chimerism and other CR-related changes may be detected here. Depending on the researcher's focus and interest, further analyses can be performed with the freshly prepared graft tissue: intestinal whole mounts for evaluation of cellular infiltrate into the tunica muscularis can be prepared [45] and also used for a standardized in-vitro measurement of graft contractility [46]. Motor graft function can be further analyzed using electrophysiologic measurements [47]. Interestingly, functional graft assessment was only performed in a minority of published studies. Nutrient absorption, a major outcome factor in intestinal transplantation, reflects mucosal graft function and can be measured both in vivo and in vitro [17,20,22,26,48].

4.3. Which immunosuppression should be used?

To prevent ACR and allow for the development CR, a standardized but time-limited immunosuppressive protocol might be advisable. Both Cyclosporin A and Tacrolimus are effective to prevent ACR in orthotopic ITX models and several protocols for immunosuppression have been proposed to induce CR. All published protocols share that, in order to induce CR, immunosuppression was withdrawn at some point. However, after a comprehensive analysis of the included studies we conclude that an ideal protocol for CR induction after experimental ITX does not exist. At present, the use of CNI-based protocols might be closest to the current standard in human ITX.

4.4. Does an ideal time point of analysis exist?

The heterogeneity of the published work on CR after experimental ITX makes it difficult to draw conclusions on the optimal time point for analysis. Yet, the pathogenesis of CR may not be fully characterized by horizontal studies looking at end-stage CR of the intestinal graft only. Longitudinal studies including earlier time points (before the full histological manifestation of CR) might be better suited to clarify CR pathogenesis, establish a predictive biomarker profile and possibly develop preventive strategies for intestinal CR.

4.5. Which factors are known to impact on CR?

From the data of the included studies, it becomes evident that several clinical conditions impact on the onset, severity and progression of CR in the transplanted intestine. Most of these rely on immunological mechanisms which can involve cellular or humoral pathways or, most likely, both. Profound ischemia and reperfusion injury is believed to trigger an inflammatory process in the intestinal graft that may not fully resolve in some instances and thus facilitate the development of CR [49]. This hypothesis is supported by the observation that a plethora of inflammatory markers such as interleukin 6 (Il-6), tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) as well as high mobility group box 1 (HMGB-1) have been described as aggravating factors of CR. The alarmin HMGB-1 is released by the extracellular matrix during cellular stress or damage and acts as an endogenous ligand of toll-like receptor 4 (TLR4), a central pattern recognition receptor with downstream proinflammatory signaling [50]. The triggered proinflammatory milieu links the innate to the adaptive immune system as Il-6 promotes Th17 generation and disfavors stabilization of newly generated T regulatory cells [51,52]. The enhanced Th1 milieu may perpetuate allorecognition pathways consequently promoting CR. Similar phenomena might apply to amplified antigenicity of heterotopic transplanted grafts with a lack of microbiome (and also TLR4)-mediated immune homeostasis. CMV infections and cellular damage with increased graft antigen exposure (for example by recurrent episodes of ACR) are hypothesized as aggravating factors in CR [35,40,53]. Whether ACR episodes shortly after ITX can lead in the contrary to clonal exhaustion with subsequent operational or true tolerance is unclear [54].

On a cellular level, CR has been noted to correlate with an increased infiltration of monocytes, $CD8^+$- and $CD4^+$ t-cells as well as NK- and mast cell infiltration into the intestinal graft [24,55,56]. However, it is not known which of these cell populations are pivotal for the processes of CR. It may be speculated that the host monocytic cell compartment could constitute one possible crosslink between limited ischemia/reperfusion-induced inflammatory changes and irreversible graft destruction by adaptive immune mechanisms. These cells are recruited into the graft and can serve as precursors of dendritic cells [57]. The monocyte-derived DC have the capability of presenting graft antigens to recipient T-cells, which would in turn explain the subsequent upregulation of an anti-donor adaptive immune response with CR as the common endpoint.

4.6. What might be interesting approaches for the future?

From the available experience, the mechanisms of CR should be ideally studied in a technically feasible model with potential for

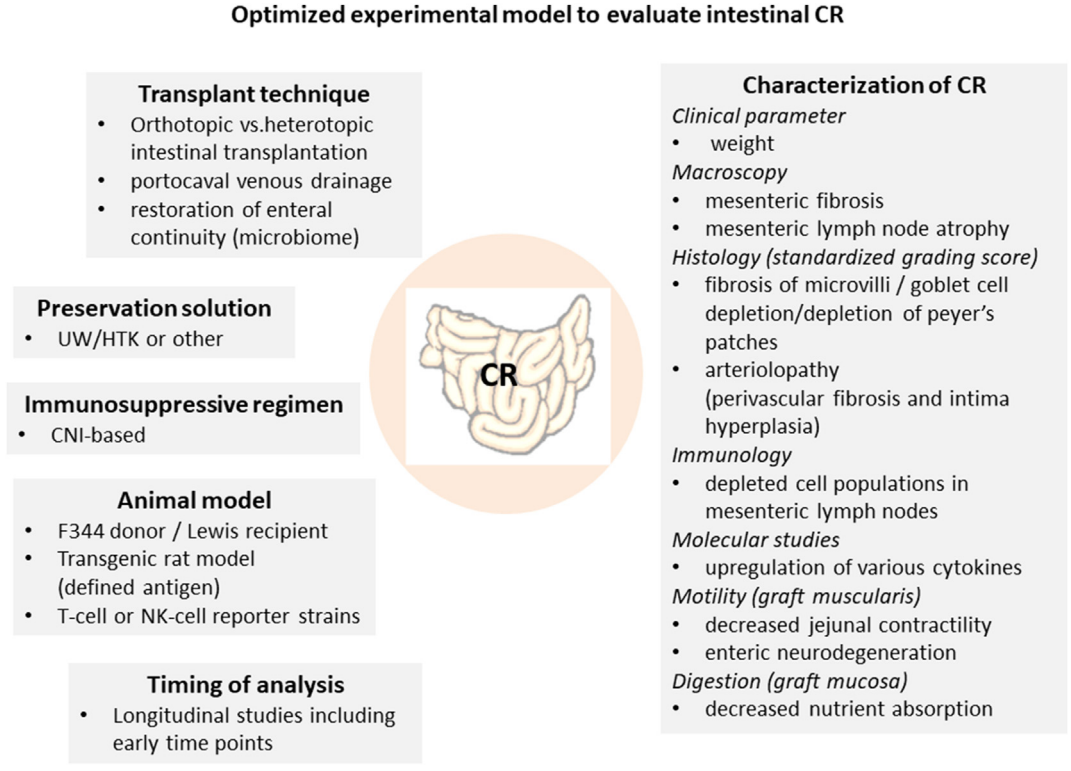

Fig. 4. Optimized experimental model to evaluate chronic intestinal rejection. Several limitations in design and reporting minimized the translational value of the historic studies on experimental CR after ITX. We propose certain parameters and considerations that might be helpful to design experiments on CR in the future.

immunologic in-depth analysis. Introducing a defined modified antigen in a transgenic rat strain would be a feasible option. With the recent advent of the CRISPR/Cas technology, the availability of such models will most likely rapidly increase and be within reach for the transplant scientist in the near future [58]. Using a predefined transgenetically introduced antigen in an otherwise inbred strain combination, antigen-specific T-cell infiltration and expansion could be easily studied using flow-cytometry. It has become possible to differentiate between donor- and recipient-derived T-cells of standard BN (RT1.Ac), F344 and Lewis rats (RT1.A1) by using specific antibodies against MHC Class I alleles [59,60], but the utilization of T- or NK-cell reporter strains would immensely simplify immunologic studies in a model of CR. As an example, in human ITX the role and relevance of humoral alloimmunity, especially by donor-specific antibodies (DSA) for the induction and progress of CR is discussed intensively, yet to our knowledge and confirmed by our search we could not identify any experimental studies that aim to mechanistically address this topic in experimental ITX. New immunologic approaches could facilitate studies to this end. Peripheral tolerance mediated by the four principles clonal deletion, ignorance, T cell anergy and active suppression is still discussed to be key to allograft tolerance [61]. Specifically for the active suppression of T cells, regulatory T cells which are phenotypically marked by the expression of the master transcription gene forkhead box 3 (Foxp3+ CD4+ CD25+ Treg) are suggested to be important for intragraft immune quiescence [62]. Foxp3 has shown potential as a surveillance marker for absence of rejection in non-intestinal solid organ transplantation in humans [63]. To our knowledge, no studies on the role of Treg in experimental CR have been attempted. Tregs have been linked to B regulatory cells, which in turn can control T-cell dependent inflammation [64]. Importantly, unregulated B cell activity with generation of preformed or de-novo donor specific antibodies has recently been identified as a risk factor for graft failure in human ITX [65]. Thus, both the role of Tregs and the aspect of antibody mediated rejection would be worthwhile study parameters in future models of experimental CR.

The "liver protective effect", in which simultaneous transplantation of the liver results in functional protection of the intestinal graft and significantly ameliorates rejection, is a clinically known phenomenon [48,66–69]. This effect has been observed in human intestinal and other solid organ transplantation but its mechanism remains unresolved [70,71]. It has been proposed that the amount of transplanted donor mass and chimerism might play a role [45]. The protective effect of immunomodulation by bone marrow transfusion in contrast to the deleterious promotion of CR by graft irradiation supports the role of chimerism for graft acceptance [72]. In the review by Lauro et al., the liver protective effect is mentioned in greater detail as a possible opportunity for research on CR prevention [2]. An experimental study in rats showed near normal architecture in the intestines of rats that had received a simultaneous liver graft as compared to isolated intestinal grafts [68], however similar to the aforementioned dearth of experiments on the effects of Tregs and humoral immunologic factors in intestinal CR, there are no ongoing studies aiming to elucidate the "liver protective effect" in an experimental setting. Hence, experimental multivisceral transplantation approaches with a focus on CR might provide valuable insights.

5. Conclusion

In summary, CR is one important cause of long-term graft failure after human isolated ITX. Historically, rodent models have been mainly used to evaluate CR pathogenesis and test therapeutic strategies. Although both innate and adaptive immune processes have been identified to be involved, no effective strategies for prevention or treatment of intestinal CR have found their way into clinical practice. After systematically reviewing the body of experimental literature on intestinal CR, we conclude that innovative new studies combining microsurgical expertise with in-depth immunologic techniques are warranted to better understand intestinal CR. We aimed to facilitate future research on CR in experimental ITX by proposing an optimised experimental model that can be used as a blueprint for further studies (See Fig. 4). Subsequent translation of new experimental knowledge into clinical practice could aid in improving long-term outcome and graft survival of human intestinal transplantation.

Disclosure

The authors have no conflicts of interest to disclose

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trre.2019.04.001.

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