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World Journal of Experimental Medicine (*World J Exp Med*, *WJEM*, online ISSN 2220-315X, DOI: 10.5493) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

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MINIREVIEWS

Animal models of *ex vivo* lung perfusion as a platform for transplantation research

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Abstract

Ex vivo lung perfusion (EVLP) is a powerful experimental model for isolated lung research. EVLP allows for the lungs to be manipulated and characterized in an external environment so that the effect of specific ventilation/perfusion variables can be studied independent of other confounding physiologic contributions. At the same time, EVLP allows for normal organ level function and real-time monitoring of pulmonary physiology and mechanics. As a result, this technique provides unique advantages over *in vivo* and *in vitro* models. Small and large animal models of EVLP have been developed and each of these models has their strengths and weaknesses. In this manuscript, we provide insight into the relative strengths of each model and describe how the development of advanced EVLP protocols is leading to a novel experimental platform that can be used to answer critical questions in pulmonary physiology and transplant medicine.

Key words: *Ex vivo* lung perfusion; Transplantation; Rat; Porcine; Small animal; Large animal; Model; *Ex vivo* lung perfusion

Core tip: *Ex vivo* lung perfusion allows for lungs to be assessed for their physiologic and functional parameters prior to transplant. As a tool for experimental research, the technology is an extremely powerful tool that enables isolated organ modification and evaluation. Utilizing small and large animal models have complementary approaches to addressing transplant related questions.

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INTRODUCTION

Overview of lung transplantation donor organ shortage

Lung transplants have become a viable option for patients with end stage lung disease. Unfortunately, only about 15% of donor lungs are deemed appropriate for transplant[1], and estimates show that about 50% of patients die while waiting for a lung transplant[2]. Addition-

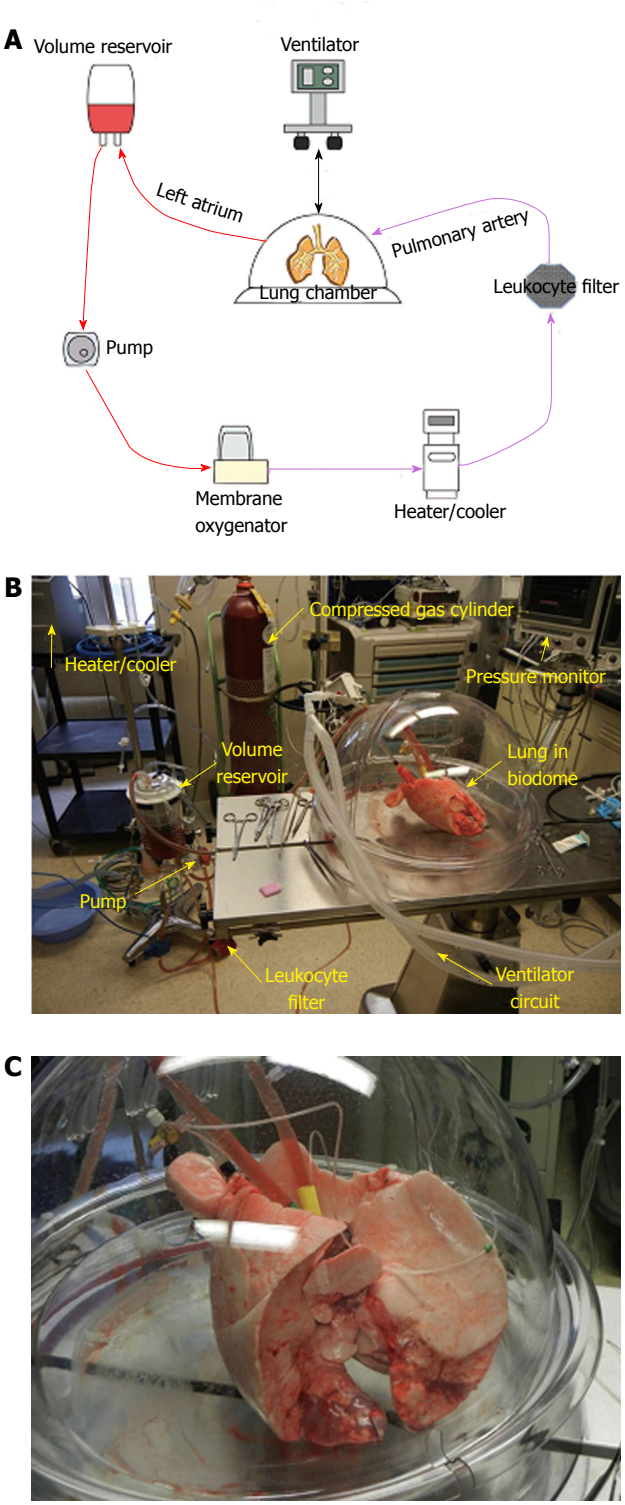

Figure 1 This diagram depicts a schematic of a large animal (porcine) *ex vivo* lung perfusion circuit (A), the portable stand for the perfusion pump (B), A close up picture of a porcine lung on an *ex vivo* lung perfusion circuit (C). The ventilator is used to expand the lungs with lung protective ventilation strategies. The volume reservoir contains the perfusates (either sanguineous or acelluar). Centrifugal pumps have the advantage of being able to have the afterload varied and have the circuit clamped easily, which is a challenge with roller pumps. The heater/cooler allows for exact temperature titration. The reservoir, centrifugal pump, membrane oxygenator, and leukocyte filter are all contained on this apparatus. The biodome which houses the large animal lung is visualized with the ventilator circuit attached to the endotracheal tube which directly cannulates the trachea. The inflow and outflow cannulas are at the superior aspect of the lung and the endotracheal tube on image top left.

ally, the average patient who receives a lung transplant waits 412 d for a suitable lung[3]. Because so many lungs do not meet transplantation requirements, the quality of available lungs must be increased in order to increase the amount of lungs available for transplant.

Ex vivo lung perfusion

Ex vivo lung perfusion (EVLP) (Figure 1) has the potential to increase the lung donor pool and allows for precise control of important variables including perfusate composition, temperature, tidal volume, positive end expiratory pressure (PEEP), fraction of inspired oxygen, and arterial pressure. EVLP can improve donor lungs that were originally thought to be in too poor a condition to be transplanted and can also be used to determine a lung's condition for donation[4,5]. EVLP also allows for the assessment of donor lungs without having to transplant them to another person.

Current clinical state of EVLP

Steen *et al*[4] first published their paper on the transplant of a lung that was perfused *ex vivo* in 2007. In 2011, Both Cypel *et al*[6] and Lindstedt *et al*[7] reported that initially rejected lungs that were perfused *ex vivo* performed similarly to lungs that were initially selected for transplant. In 2012, both Aigner *et al*[8] and Zych *et al*[9] reported that EVLP has the potential to improve the quality of donor lungs that otherwise would not be selected for transplant. In 2013, Wallinder *et al*[10] reported the EVLP is a safe method and allows lungs that would have been rejected to be used in transplants. The potential impact of EVLP to expand the available organ donor pool is profound. If the lung donor conversion rate is able to be increased from 17% to 30%, that small incremental increase in donor conversion would ostensibly double the number of transplants able to be performed worldwide annually. Evaluating the mechanisms of lung injury and progression would enable targeted therapies to intervene on these critical set points. EVLP provides an isolated platform where these mechanical traumas can be isolated and evaluated in a mechanistic fashion. Through a combination of lung protective ventilation, reducing airway edema, and targeted therapies we would anticipate that the increase conversion rate would be able to be met.

EVLP AS AN EXPERIMENTAL PLATFORM

Evaluation of organ function

While performing EVLP, multiple factors can be assessed in real-time to determine the viability of the lung. These include pulmonary arterial flow, pulmonary arterial pressure and pulmonary resistance, as well as dissolved oxygen concentration in the perfusate before and after passing through the pulmonary circulation. This change in dissolved oxygen corresponds to the oxygen production by the lung. The wet-to-dry ratio of a lung can also be assessed, giving an accurate depiction of how edematous the lung has become.

Table 1 Dependent and independent variables with *ex vivo* lung perfusion

Dependent variables (*i.e.*, what can be measured with *ex vivo* lung perfusion)	Independent variables (*i.e.*, what can be varied in an *ex vivo* lung perfusion)
Tracheal pressure	Tracheal pressure
End expiratory pressure	End expiratory pressure
End inspiratory pressure	End inspiratory pressure
Tidal volume	Tidal volume
Compliance	Respiratory rate
Respiratory rate	Pulmonary artery flow rate
Pulmonary artery flow rate	Pulmonary artery pressure
Pulmonary artery pressure	Left atrial outflow pressure
Left atrial outflow pressure	Perfusate
Pulmonary vascular resistance	Ischemic time
Lung weight	Temperature of perfusate
Wet to dry ratio	Temperature of organ
Pre-organ pO_2	Inspired gas concentration and components
Post-organ pO_2	
Oxygen production	
Perfusate pH	
Perfusate pCO_2	
Perfusate for molecular analysis	
Tissue for mRNA, protein, or histologic analysis	

Model of acute lung injury development

EVLP can also be used as a model of acute lung injury (ALI) development and ventilation induced lung injury (VILI). Currently, there has been only one clinical trial that has resulted in a significant decrease in patient mortality related to ALI and VILI[11]. Although multiple ventilation protocols have been researched, there is little information on how drug treatment might affect lung viability at various tidal volumes and positive-end expiratory pressures. Multiple models of ALI that are typically used for *in vivo* studies (*i.e.*, saline lavage/surfactant dysfunction, acid induced lung injury and LPS induced lung injury) can be easily and quickly implemented using EVLP (Table 1). In addition, precise regulation of tidal volume, PEEP and other ventilator parameters during EVLP allow for modeling the mechanically induced injury that occurs during VILI. Unlike *in vivo* models, EVLP models of ALI/VILI allow for the evaluation of how specific ventilator settings influence lung injury progression without the confounding effects of changes in other physiologic parameters (Table 1). These models can be assessed by measuring pro-inflammatory cytokine secretion and histological characterization of lung tissue. The lungs can also be treated with specific drugs delivered through the perfusate or trans-trachealy to determine if any drug combination results in a minimization of lung damage during ventilation.

Pathway to evaluate efficacy of experimental treatments

Perfusate: The selected perfusate should have osmotic and oncotic pressures similar to blood and must also provide an energy source for the cells. Clinically, the perfusate is used to evenly cool the organ tissue and to remove blood, thereby preventing cell injury. It is important to note that the perfusate and all of its components have direct contact with the perfused organs and therefore are an extremely important variable in determining the outcome of EVLP.

Steen *et al*[4,5,12] developed a new perfusion solution and proved EVLP to be a viable method to improve and preserve donor lungs, and it continues to be the most popular perfusion solution used. The Pego-Fernandes group reported that their solution, low potassium dextran-glucose (LPDnac) was comparable to Perfadex (Vitrolife, Goteborg, Germany) but found saline to be inadequate[13,14]. Menezes *et al*[15] also compared Perfadex to Celsior and found lungs perfused with either exhibited similar gas exchange and histopathological findings.

Gene or molecule delivery: Multiple groups have shown that gene therapy coupled with EVLP can repair injured lungs before transplantation. Cypel *et al*[16] demonstrated that the delivery of adenoviral vector encoding human interleukin-10 (AdhIL-10) to human lungs showed improvement in arterial oxygen pressure and vascular resistance, concluding that delivery of AdhIL-10 can improve lung function. Yeung *et al*[17] later showed that ex vivo delivery of adenoviral genes to lungs is superior to *in vivo* delivery due to the decreased vector-associated inflammation and improved post-transplant lung function. Emaminia *et al*[18] also showed that delivery of adenosine A2a in the perfusate reduced the inflammatory response in acutely injured pig lungs.

Optimize the nutrients needed to sustain the lungs: Using an acellular perfusate can avoid mechanical damage to the lung over long lung perfusions[1,19] and is more widely used over cellular solutions. Pro-inflammatory cytokines can accumulate in the perfusate over time so the perfusate should be replaced periodically to avoid increased inflammation.

Trans tracheal and aerosolized agent delivery: Drugs cannot only be delivered through the perfusate, but also as an aerosolized drug trans-tracheally. Pulmonary delivery of aerosolized drugs has been modeled using an EVLP system by many groups. Dong *et al*[20] showed that administration of aerosolized chitosan-coated poly (lactide-co-glycolide) based nanoplexes containing antisense 2'-O-Methyl RNA (OMR) resulted in a significantly higher uptake of OMR in the respiratory epithelium compared to administration of OMR alone using an EVLP model. Beck-Broichsitter *et al*[21] also used an EVLP model to show that delivery of biodegradable nanoparticles may be a viable approach for drug delivery.

LARGE ANIMAL MODEL OF EVLP

Advantages

Porcine EVLP has a direct translation to the human clinic (Figure 1). In general, the advantages of this large animal model of EVLP can be broadly grouped into the following 4 categories.

Table 2 Physiologic *ex vivo* lung perfusion parameters

	Rat	Pig
Tidal volume	4-10 mL/kg	6-8 mL/kg
Positive end expiratory pressure	2-6 cm H_2O	5 cm H_2O
Flow rate	5-30 mL/min (estimated cardiac output: 25-50 mL/min per 100 g)	40% cardiac output/min (estimated cardiac output: 100 mL/min per kilogram)
Pulmonary artery pressure	13.6 cm H_2O	10-15 mmHg
Perfusate albumin concentration	2%-4%	5%-7%

Size appropriate: The swine model offers very appropriate size comparisons to humans. Because of this, comparable tidal volumes, PEEP, and perfusion times can be used for the EVLP. As a result, information obtained in this large animal model of can be rapidly and directly transferred to settings for clinical trials. This direct transfer of information to clinical trials is typically not possible when using smaller animal models. There are variations in physiologic parameters based on animal model sized (Table 2).

Similar immune system and biology: The pig has a greater similarity to humans in gene sequence and physiology compared to mice and rats which makes it a superior model[22]. This results in a simpler comparison to humans and therefore a more direct path to clinical relevance.

Allows for opportunity to perfect scale up to human size and clinic setting: Because of the pig's larger size, the opportunity exists to experiment with the exact same equipment that would be used in clinical trials[12]. The amounts of perfusate needed as well as ventilator settings are more closely related to clinical settings compared to smaller animal models. The amount of time a pig lung can be perfused is comparable to humans.

Accepted transplant model: All animals should receive care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research, the Guide for the Care and Use of Laboratory Animals.

Generally, protective settings for mechanical ventilation are used during EVLP. Typically, pigs are sedated with 40 mg/mL ketamine and anesthetized with 8 mg/kg pentobarbital. A period (about 60 min) of warm ischemia is usually implemented before starting EVLP to mimic a donor's lung. Volume-controlled ventilators are used and tidal volumes of about 4-6 mL/kg, a PEEP of about 5 cmH_2O, and a respiratory rate of about 17 breaths per minute are used. The fraction of inspired oxygen (FiO_2) usually ranges from 100% to 40% (Table 2).

Limitations

While pigs offer one of the best parallels to humans, their cost to purchase and to care for is much higher than small animals. The amount of perfusate used on an isolated pig lung is much higher than with small animal, making each experiment much more expensive. This also makes having a high amount of replicates in an experiment very difficult. Because of the large size of an isolated pig lung, the perfusion circuit itself is custom built and requires the same equipment that would be used for a clinical perfusion.

SMALL ANIMAL MODEL OF EVLP

Overall advantages

Small animal models that have been employed in EVLP include rat, mouse, guinea pig and rabbit (Figure 2). These systems offer several distinct advantages compared to their larger counterparts. Overall, their cost is much lower. This includes initial startup cost, such as in surgical and perfusion equipment as well as the animals themselves. Because of the smaller cost, one can complete more perfusion experiments with less money and in less time than if the same study were completed using a porcine or canine model system. Additionally, one can capitalize on the higher sample size in order to aid in achieving statistical significance. Most small animal experiments use 5-8 animals per group and up to 50 animals in total per study[23-26]. These numbers are simply not feasible in larger systems and helps increase confidence in experimental data.

An inherent advantage of EVLP is the isolation of the lungs from the rest of the body. This has helped elucidate differences in the immune response of resident lung cells compared to the systemic immune response during ischemia/reperfusion (I/R) in a mouse model[26,27]. More generally, this characteristic of EVLP can be exploited to more easily vary experimental components and limit confounding factors. One avenue of research that has been pursued extensively is in the optimization lung perfusate solutions. This is an area of critical importance in the development and refining of EVLP procedures for clinical use and is a current topic of controversy.

Basic properties, such as the optimal electrolyte composition of the perfusate itself are not agreed upon. Current data are unclear as to which currently available solutions perform best[15]. Perfadex, a solution developed specifically for lung preservation, may not offer better preservation than Celsior, a heart preservation solution[15,25]. One group in Brazil compared Perfadex to a locally produced generic solution, LPDnac and found it to preserve lungs just as well[13]. The potential benefits of varying perfusate temperature and introducing vasodilators has also been studied[28]. Despite the disagreements over perfusate composition, small animal EVLP systems provide an excellent platform for further perfusate development and testing.

Rat/rabbit/guinea pig models

Of the different small animal systems used for EVLP experiments, each offers their own advantages and draw-

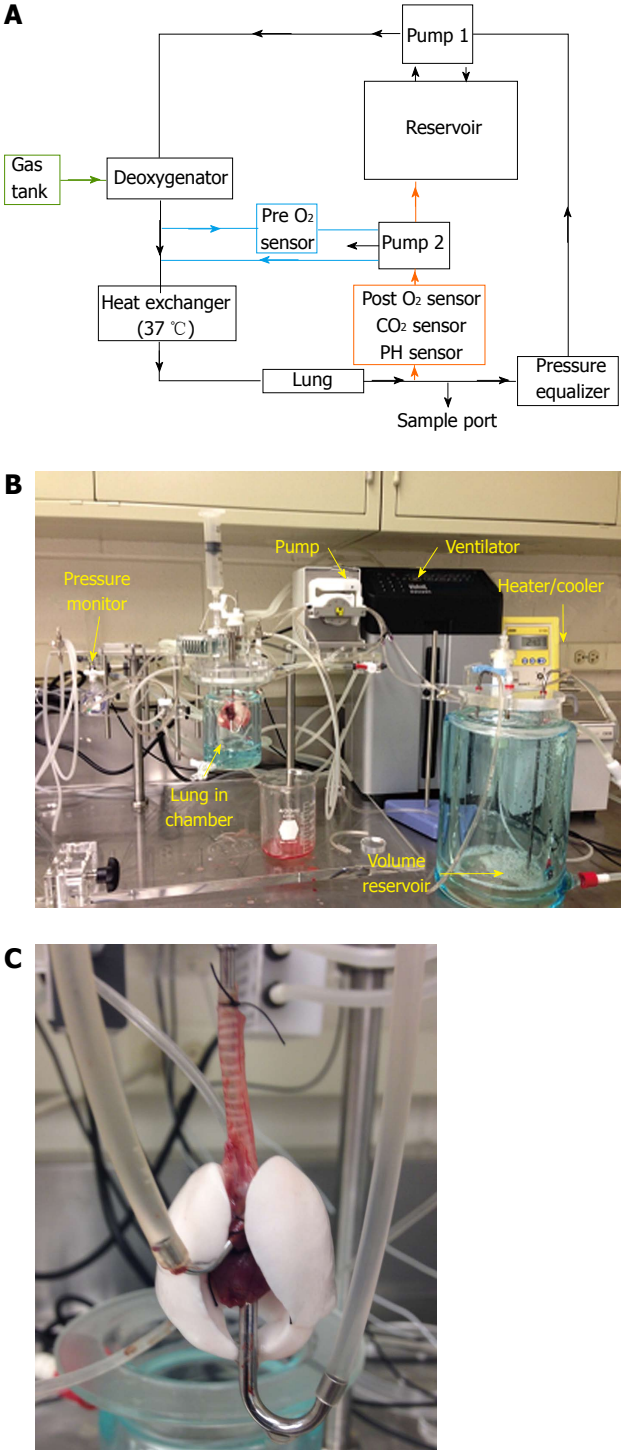

Figure 2 This diagram depicts a schematic of a small animal (rat) *ex vivo* lung perfusion circuit (A), the small animal perfusion circuit (B); a close-up of a rat lung undergoing *ex vivo* perfusion (C). Many of the same characteristics that are in the large animal circuit are present. This particular circuit has the ability for fine measurements of pressure, flow, and weight. The image back right shows the thermoregulator and the ventilator. The perfusates reservoir is in the image front right. The small animal circuit is analogous to the large animal circuit. However, due to the relative scale of the organ to the circuit, the perfusate volume needed for a complete perfusion is less. In addition, the ability to perform positive as well as negative pressure ventilation is possible. This varied ventilation an mimic both the mechanical breathing as well as natural intrathoracic breathing. The tracheal cannulation is top-center. The inflow cannula going into the pulmonary artery is from top-left and the outflow cannula going across the left atrium through the left ventricular apex is on screen right.

backs. Rat, guinea pig and rabbit models have a larger thoracic cavity than mice, making surgical procedures easier. Owing to their larger size, initial cannulation (Figure 2) is relatively simple and can be done with or without the aid of a surgical microscope[29,30]. Moreover, a rat left lung transplantation (LTX) technique has been developed and used in multiple studies[29-32].

Recent improvements have increased the success rate of this LTX technique to greater than 95%[29]. Inokawa *et al*[32] used this procedure to create a specific model of transplantation as it relates to EVLP and designed it to closely mimic clinical conditions. Rat donor lungs are explanted, stored on ice for 1 h, perfused, stored on ice again for 2.5 h and finally transplanted. This model has been used to demonstrate the therapeutic potential of low concentration carbon monoxide ventilation during perfusion[23]. Although less common, rabbit[33] and guinea pig[34] models have been used to study the onset of ischemia-reperfusion injury.

One challenge, however, with the use of these three animals as model systems is the relative scarcity of species-specific commercially available antibodies and molecular reagents. Because of this, protein studies are limited in these systems, though Fehrenbach *et al*[35] demonstrated in a rat model of EVLP that the concentration surfactant protein A (SP-A) increased following I/R using a polyclonal antibody against SP-A.

Murine models

Murine models of EVLP offer considerable advantages over rats because of the greater number of species-specific antibodies and gene probes available for experiments. This has facilitated development of a much greater body of scientific literature with regard to these types of studies. For example, the murine immune response to EVLP has been studied for over 15 years[36]. More recently, Barrenschee *et al*[37] used toll like receptor (TLR) agonists to mimic the response during infection and characterized levels of key cytokines/chemokines such as interleukin (IL)-1β, IL-6 and TNF-α. Siegl and Ulrig studied the inflammatory response of mice in high and low ventilation scenarios, including quantification of the phosphorylation of key enzymes involved in the inflammatory response[38].

An additional advantage of the mouse model is the availability of knock out (KO) lines. Deficient genes could be related to the inflammatory response, including TLR-4 deficient[39] and TNF-α deficient mice[27] or could interfere with other areas of lung function[40]. Maxey *et al*[27] used the TNF-α deficient mice in EVLP to demonstrate the importance of TNF-α in initiating the inflammatory response following I/R.

Recently, a model of mouse lung transplantation has been developed for further study of obliterative bronchiolitis. The procedure is very similar to the rat model of LPX from a technical standpoint, but to our knowledge, has not yet been used as an EVLP model[41,42]. This may be due to increased technical difficulties during mice op-

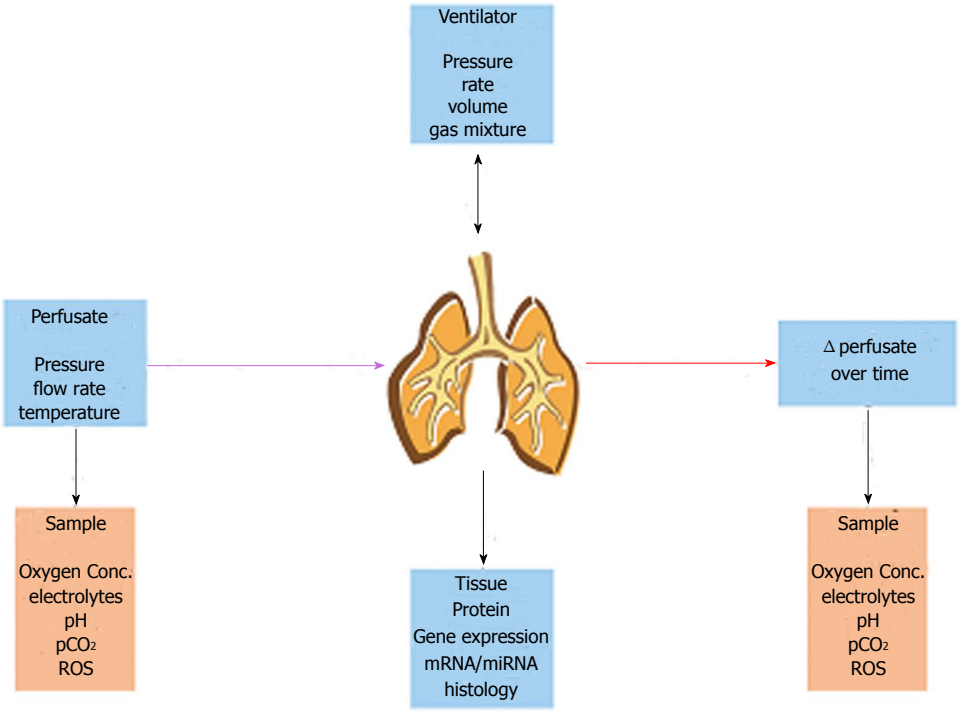

Figure 3 Diagram of what is able to be able to be measured and varied with the *ex vivo* perfusion circuit. This figure directly correlates with Table 1.

erations because of their smaller size. However, it is likely that once some initial sets of experiments combine this mouse LTX technique with EVLP, the scope of possibilities of what EVLP platforms can study will be widened.

The greatest challenge in solely relying on the murine model of EVLP is the technical difficulties involved during surgery. Mice have a smaller thoracic cavity and smaller organs than rabbits, rats or guinea pigs. Often, a surgical microscope is required to identify and isolate key structures during the heart-lung block explant[26,27]. Another drawback of this mouse model is the increased difficulty of training personnel on more technical mouse surgery procedures, which can create bottlenecks in experimental plans and ultimately slow down data acquisition. For this reason, it is likely that future studies will still utilize all small animal models, with mouse models of transplant used when necessary (for protein and gene studies) and lung mechanics studies primarily completed using a rat model.

Limitations

Owing to their small size and cost effectiveness, small animal models of EVLP are extremely convenient. When considering their use, however, several key differences need to be taken into account. Mice and rats have much shorter perfusion times than human or pig lungs. One rat model of lung transplant includes 15 min of perfusion time[32]. Other studies perfuse for 50 min[15,25,35] or 60 min[15]. One needs to keep in mind the mismatch in times scales, as murine lungs after 15 min of perfusion/ventilation are closer in damage to pig/human lungs perfused for a much longer time (4-24 h depending on the lung injury model being studied).

Yet another difference is that rodent lungs are significantly more susceptible to atelectasis. As a result, during the "ischemic" periods of a mouse model of EVLP, the lungs are still ventilated, albeit at a lower rate and in a hypoxic environment[26,27] This is unavoidable though, since without ventilation the lungs would not remain viable long enough to complete the study. Previous investigators have demonstrated that atelectasis and the subsequent reopening of fluid occluded regions can damage the lung epithelium[43,44] and exacerbate inflammation[45,46]. Therefore, it is extremely important to prevent lung damage, atelectasis and pulmonary edema because, unlike human and large animal models, a bronchoscopy cannot be performed to clear fluid from the lungs. Assuming all of these major differences are taken into account, small animal models are excellent starting points for the development of EVLP for clinical use and for the testing of therapeutics against I/R injury.

TECHNICAL CONSIDERATIONS

Perfusate

Steen solution is the most popular solution used to date and acellular solutions are much more common than cellular solutions. Studies indicate a hyper-oncotic, albumin-based solution is best. The acelluar solutions have the potential benefit of not adding an exogenous antigen source and the red cells are not lysed through the mechanics of the perfusion. The acellular solutions have the potential benefit of helping to support metabolic demands. In the lung this is not as critical as in other organs since the lung itself provides the oxygen. The perfusate needs to be buffered and provide glucose and electrolytes.

Ventilator settings

Ventilator settings should be protective during EVLP for best results. In the large animal model this means a tidal volume of 4-6 cc/kg. From time to time, 10 cc/kg is used. In the rat model, a protective tidal volume is 4 cc/kg with 10 cc/kg being potentially deleterious. Depending on the hypothesis being tested and the animal model used, multiple variables can be changed on the ventilator including tidal volume, PEEP, breaths per minute, and fraction of inspired oxygen (Figure 3).

Temperature

Perfusate temperature is usually either increased temporally or based on current temperature. The perfusate temperature is usually increased until 37 ℃ is achieved. An in-line thermoregulator or perfusion heater/cooler is used to titrate the temperature. A cold or warm ischemic period may precede the actual perfusion depending on the hypothesis being tested.

Duration of perfusions

Small animal perfusions usually run between 30 min-3 h. Pig EVLP have been run for up to 14 h. The times vary greatly depending on the animal model used and the hypothesis being tested.

Pulmonary artery flow rates and pressures

Perfusate flow rates are usually set to achieve a specific pulmonary pressure or a specific pulmonary resistance. A typical experimental set-up is to have the perfusion flow rate increase incrementally over the duration of the perfusion (15-30 min time period). Once full flow (40% cardiac output) is achieved, the pulmonary artery and left atrial pressures are measured. The pulmonary vascular resistance is calculated as a function of the pressures and flow rates. In a well-functioning organ, pulmonary vascular resistance decreases over time. In a poorly functioning organ, the resistance increases. Increased resistances often mirror poor oxygenation.

CONCLUSION

EVLP has great potential to increase the lung donor pool by providing a platform for improving and evaluating lungs initially thought to be inadequate. Multiple groups across the globe are developing promising models to achieve a greater donor pool. EVLP is also being used as a model for acute lung injury to better understand how the complex mechanical forces applied to the lungs influence injury development and inflammation and to develop strategies that limit the amount of tissue damage/inflammation. EVLP is also being explored as an opportunity for administering therapeutic agents. This idea is unique in that it bypasses the patient's immune system and allows for a higher acceptance rate compared to drugs administered *in vivo*.

Both small and large animal models are advancing our knowledge on EVLP and each has their own specific advantages and disadvantages. While small animal models do not usually run for more than 1-2 h, they are economical and allow for many experiments in a short period of time. Swine models are very expensive but allow for the closest model to human lungs available and use the same equipment that would be used clinically. Since nearly 50% of patients die while waiting for a lung transplant, it is crucial to expand the donor pool. EVLP holds the most promise towards achieving this goal.

The ability to keep organs alive and perfused for extended periods of time will enable the "culture" of organs. This prolonged, perfusion will be the basis for immunomodulation and change of the endothelium through nanoparticle, gene bases, or antibody based delivery of therapeutic agents. This will be the dawning of customized medicine to tailor the transplanted organ to the individual recipient and their biology.

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MINIREVIEWS

Wound healing reaction: A switch from gestation to senescence

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Abstract

The repair of wounded tissue during postnatal life could be associated with the upregulation of some functions characteristic of the initial phases of embryonic development. The focusing of these recapitulated systemic functions in the interstitial space of the injured tissue is established through a heterogeneous endothelial barrier which has excretory-secretory abilities which in turn, would induce a gastrulation-like process. The repair of adult tissues using upregulated embryonic mechanisms could explain the universality of the inflammatory response against injury, regardless of its etiology. However, the early activation after the injury of embryonic mechanisms does not always guarantee tissue regeneration since their long-term execution is mediated by the host organism.

Key words: Wound healing; Repair; Embryonic mechanisms; Vitelline; Amniotic

Core tip: In this review, we propose an integrative molecular point of view about wound healing. Wound healing could be associated with the upregulation of functions characteristic of embryonic development. The repair of adult tissues using upregulated embryonic mechanisms could explain the ubiquity of the inflammatory response against injury, regardless of its etiology.

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INTRODUCTION

Wound tissue repair can be realized by regeneration and/or fibrosis. While regeneration describes the specific substitution of the injured tissue, tissue fibrosis displays an unspecific form of healing in which the wounded tissue heals by scar formation[1,2]. Since repair by fibrosis can be considered an unsuccessful attempt of wound tissue repair by regeneration, the fibrotic process supposedly represents an insufficient repair method and, therefore, a pathological response. This is the reason why the inflammatory response associated with scar formation is also commonly labeled pathological. In this way, regenerative healing has a notable absence of inflammatory cell activity[3-5]. Consequently, inflammatory response mediators have been a focus of investigation in studies aiming to curtail scarring[5,6].

The standard view of inflammation as a reaction to injury or infection might need to be expanded to account

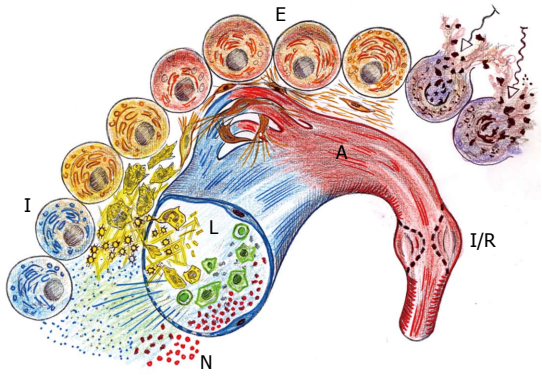

Figure 1 Schematic representation of the different stages of wound repair. During the post-traumatic local inflammatory response three successive and overlapped phases: in the arterial side of the microcirculation (red), a nervous (N) or immediate phase with ischemia-reperfusion (I/R) occurs; in the post-capillary venule (blue), an immune (I) or intermediate phase with a leukocytic (L) phenotype is expressed; and, finally an endocrine (E) or late with an angiogenic (A) phenotype is developed, which implies the capillaries neoformation.

for the inflammatory processes induced by other types of adverse conditions[7]. The human diseases that are associated with these conditions, including atherosclerosis, asthma, type 2 diabetes and neurodegenerative diseases, are all characterized by chronic low-grade inflammation[7]. However, human aging can be explained by the emerging concept of inflamm-ageing, *i.e.*, - a combination of inflammation and aging[8]. Inflamm-ageing seems to favor the onset of typical age-related diseases like atherosclerosis, dementia, osteoporosis and cancer[9]. Inflammatory mechanisms are also involved in physiological processes, like physical exercise, embryonic development and gestation, and indeed there is the hypotheses that the evolution of the living species could be based on inflammatory remodeling of organisms induced by environmental factors[10]. It has also been proposed that, although fibrosis is often initially linked to a strong inflammatory response, there are specific mediators and pathways contributing to the pathogenesis of fibrosis that are distinct from the mechanisms driving inflammation. Thus, it is assumed that to design effective therapy for fibrotic diseases, we need to begin viewing fibrosis as a pathological process distinct from inflammation[11].

PHASES OF THE SKIN WOUND HEALING REACTION

The multiple pathophysiological mechanisms that overlap during the progression of the skin wound healing reaction may explain the lack of consensus on the number of phases involved in this reaction. Thus, the common description of the wound healing evolution includes three classical stages: the inflammatory phase to contain the injury and prevent infection; the proliferative phase characterized by new tissue formation, *i.e.*, granulation and epithelial tissues; and the remodeling phase with extracellular matrix reorganization[4,12]. However, some authors describe four healing phases: hemostasis and coagulation, with the formation of a provisional wound matrix; inflammation with neutrophil and monocyte recruitment; proliferation and repair, with the formation of granulation tissue and the restoration of the vascular network, as well as re-epithelialization; and remodeling that occurs from day 21 to up to 1 year after injury. In this phase, collagen Ⅲ, which was produced in the proliferative phase, is now replaced by collagen I and the acute wound metabolic activity slows down and finally stops[1,13]. Additionally, five phases of the wound healing reaction have also been described: hemostasis; inflammation; cellular migration and proliferation; protein synthesis; and wound contraction and remodeling[14].

In the above-mentioned descriptions of the wound healing reaction, the role attributed to inflammation is very limited and noteworthy. On the contrary, we have proposed an inflammatory etiopathogenic hypothesis of the wound healing evolution. According to this idea, inflammation could be the basic mechanism that drives the nature of the different stages of wound repair[15]. Likewise, inflammation could facilitate the integration of the pathophysiological mechanisms involved in the different phases of wound repair by scar formation[15,16]. In essence, the post-traumatic local acute inflammatory response is described as a succession of three functional phases of possible trophic meaning to the wounded tissue: nervous or immediate with an ischemia-reperfusion phenotype; immune or intermediate with a leukocytic phenotype; and endocrine or late with an angiogenic phenotype[15,16] (Figure 1).

In turn, we have suggested that these phenotypes could represent the expression of trophic functional systems of increasing metabolic complexity[17]. Therefore, it could be considered that, after the injury, the metabolic ability of every phenotype would be conditioned by the biochemical mechanisms used to provide the energy sources for cell functions[15,17]. These three inflammatory phenotypes hypothetically expressed in the traumatized tissue during tissue repair by scarring could help to integrate the etiopathogenic mechanisms expressed in each evolutive phase. In this way, these inflammatory phenotypes would associate the genetic factors, upregulated and/or downregulated, with metabolic, functional and histological alterations[17].

The interstitial space is the battle field where the inflammatory response takes place. In the successive phases of the inflammatory response, the interstitial space of the injured tissues is successively occupied by molecules, inflammatory cells, bacteria and finally by a mesenchymal-derived tissue, the granulation tissue. In summary, the inflammatory response could be viewed as a series of three overlapping successive phases with increasingly complex trophic functional systems for using oxygen since it evolves from ischemia to neovascularization[15,17].

The first or immediate phase has been referred to as the nervous phase because sensory (stress, inflammatory, pain and analgesia) and motor (contraction and relaxation) alterations, including vasomotor changes, respond

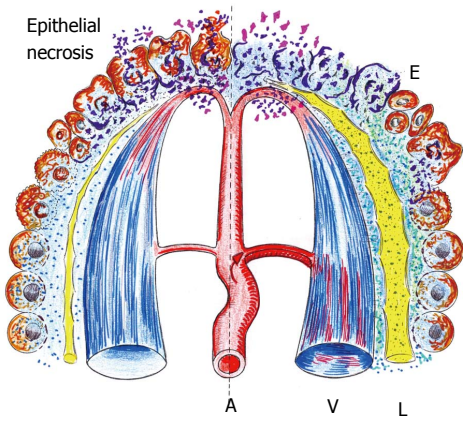

Figure 2 First or immediate phase of the acute inflammatory response. On the left side, a schematic representation in which the tissue suffers the injury and therefore necrosis of the epithelial cells are produced. In turn, on the right side, the beginning of the tissue inflammatory response in response to necrosis is shown. This initial phase presents ischemia-reoxygenation and interstitial edema (E) with interstitial infiltration of mediators of the stress response as well as substrates including glucose, amino acids and lipids. In addition, the lymphatic circulation (L) is activated. A: Arterial microcirculation; V: Post-capillary venous circulation.

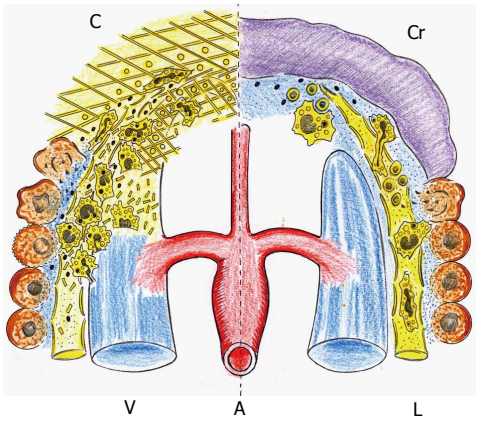

Figure 3 Immune or intermediate phase of the post-traumatic acute inflammatory response. Interstitial infiltration by platelets and leukocytes, all of them entrapped in the provisional extracellular matrix (left). Underlying the wound crust (Cr) that is formed later, the leukocytes change their phenotype to promote the resolution of the inflammatory response and wound repair by re-epithelization and scar formation (right). C: Coagulation with fibrin-platelet clot. A: Arterial microcirculation; V: Post-capillary venous circulation; L: Lymphatic circulation.

to the injury. This early pathological activity of the body's nociceptor pathways is associated with stress through the hypothalamic-pituitary-adrenal and sympathetic-adrenal medullary axes, the sympathetic nervous system and the renin-angiotensin-aldosterone system. This initial phase presents ischemia-reoxygenation, oxidative and nitrosative stress, and interstitial edema with selective interstitial infiltration by mediators of the stress response, such as catecholamines, adrenocorticotrophic hormone, glucocorticoids and angiotensin, as well as glucose, amino acids and lipids, all of them derived from earlier metabolic alterations, including hyperglycemia, protein catabolism and lipolysis. In addition, interstitial edema favors nutrition by diffusion through the injured tissue and activation of the lymphatic circulation (circulatory switch)[2,15,17] (Figure 2).

In the succeeding immune or intermediate phase of the acute inflammatory response, the wounded tissue that has previously suffered ischemia-reperfusion is infiltrated by inflammatory cells and sometimes by bacteria. This phase presents enzymatic stress with migration of macrophages and dendritic cells to lymph nodes, where they activate T and B cells, *i.e.*, innate and adaptive immune response. Interstitial invasion by leukocytes would create a new trophic axis.

Accumulating evidence demonstrates that platelets contribute to the initiation and propagation of the inflammatory process. These cells are replete with secretory granules, α-granules, dense granules and lysosomes. Platelet α-granules influence inflammation both by expressing receptors that facilitate adhesion of platelets to other vascular cells (*e.g.*, P-selectin) and by releasing a wide range of chemokines, among which CXCL4 and CLXL7 are the most abundant. Also, platelet α-granules contain a variety of both pro- and anti-angiogenic proteins. Growth factors stored in α-granules include vascular endothelium growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), hepatocyte growth factor and insulin-like growth factor (IGF). Platelet dense granules, on the other hand, contain high concentrations of low molecular weight compounds that potentiate platelet activation (*e.g.*, Adenosine diphosphate, serotonin and calcium[18,19] (Figure 3).

In the post-traumatic local inflammatory response, the activation of the innate immune system is not only based on the recognition of danger signals or danger-associated molecular patterns (DAMPs), but also relies on the presence of pathogen-associated molecular patterns (PAMPs)[20]. DAMPs and PAMPs are recognized by pattern-recognition receptors (PRRs) that are either cytoplasmic, membrane-bound or secreted. The most intensely studied PRRs are the Toll-like receptors (TLRs), in addition to innate immune receptors, the nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs)[21]. In particular, NLRs form central molecular platforms that organize signaling complexes, such as inflammasomes and NOD signalosomes. The term inflammasome was coined to describe the high molecular weight complex that activates inflammatory caspases and cytokine interleukin-1 (IL-1)β[22]. All these receptors activate signaling cascades that is based on enzymatic intra- and extra-cellular digestion[15,17] and lead to activation of mitogen activated protein kinases and nuclear factor kappa B (NF-κB)[21,22]. Once activated, TLRs induce different signaling cascades depending on the adaptor protein, ultimately leading to the activation of the transcription factors NF-κB, AP-1 and interferon-regulatory factor[22]. The regulatory event of NF-κB activation is the phosphorylation of inhibitor of kappa B kinase complex (IKB) proteins by the IKB kinase

complex, which leads to IKB protein ubiquitylation and subsequent degradation. This results in the release of cytoplasmic NF-κB complexes, which then translocate to the nucleus and drive the expression of target genes[23]. Thus, the expression of inducible genes leading to the synthesis of cytokine receptors, adhesion molecules and autacoids in the traumatized tissue is induced[24].

Leukocytes transverse the subendothelial basement membrane during their immunological surveillance patrol through tissues. This process, called diapedesis, is strongly enhanced under the influence of inflammation. The preferred extravasation sites of leukocytes are the venules[25]. Immediately after injury, extravasated neutrophils are entrapped in the fibrin-platelet clot. In the interstitium, the recruited and activated neutrophils begin the debridement of devitalized tissue and attack infectious agents. To perform this task, they release a large variety of active antimicrobial substances (ROS, cationic peptides, eicosanoids) and proteases (elastase, cathepsin G, proteinase 3 and urokinase-type plasminogen activator)[12]. Neutrophils also store pentraxins 3 and release it in response to inflammatory signals because it is an acute phase reactant[26] (Figure 3).

As monocytes extravasate from the blood vessel they become activated and differentiate into mature tissue macrophages. This transformation implies major changes in gene expression and cell function. The differential activation of macrophages is involved in many facets of tissue injury and inflammation. M1 macrophages express pro-inflammatory cytokines, such as IL-1, IL-6, IL-23 and interferon (IFN)-γ, as well as reactive oxygen and nitrogen species, which are involved in phagocytosis and the killing of microbes. They also promote type I immune responses[27]. M2 or alternatively activated macrophages fail to express pro-inflammatory mediators and are involved in angiogenesis, tissue remodeling and the resolution of inflammation. Therefore, they are supposed to promote repair functions[12,27]. T-helper cells play critical roles in modulating the differential activation of type 2 macrophages. T-helper (Th1) cells produce pro-inflammatory cytokines, *i.e.*, IFN-γ and TNF-α, which skew macrophages into the M1 phenotype. In contrast, type 2 T-helper (Th2) cells produce IL-4, IL-5, IL-13 and IL-10, which are responsible for inducing the alternatively activated macrophages or M2 macrophages[28]. Finally, it has been speculated that metabolic changes in the local milieu may program dendritic cells and other innate cells at the site of inflammation to induce a heterogeneous Th2 response[29]. Although neutrophils, macrophages and T lymphocytes are considered central in the pathogenesis of post-traumatic inflammation, recent studies also imply the involvement of mast cells and B lymphocytes as modulators of the inflammatory response and wound healing[12,30].

In the final and lasting phase of the wound healing reaction, the angiogenic phenotype is predominant because angiogenesis permits numerous substances, including hormones, to be transported by the blood circulation. Angiogenesis is based on endothelial sprouting or intussusceptive (nonsprouting) microvascular growth[31]. However, angiogenesis can also result from the recruitment of several cell populations or selected subpopulations of bone marrow-derived endothelial progenitor cells[32]. Angiogenesis is regulated by numerous "*classic*" factors, including VEGF, FGF-2, transforming growth factor (TGFs) angiopoietins, PDGF, thrombospondin-1 and angiostatin. Non-classic endogenous stimulators of angiogenesis include erythropoietin, angiotensin Ⅱ, endothelins, adrenomedulin, adipokines, neuropeptide-Y, vasoactive intestinal peptide and substance P[31]. VEGF and FGF-2 occupy the center stage in the angiogenesis field. They act in synergy to stimulate endothelial cell function during angiogenesis in tissue repair[33]. In this last phase, the endocrine phenotype favors nutrition mediated by the blood capillaries. Through initial and excessive proliferation, the endothelial cells could play a key role in the previous phase as antioxidant and anti-enzymatic cells, including induction of the acute phase response, considered the humoral arm of innate immunity[15,16]. Angiogenesis is closely associated with granulation tissue formation and remodeling. As granulation tissue forms in the healing wound, the vascular cells intermingle with the provisional matrix, which is composed mainly of fibrin, fibronectin and vitronectin[33]. Then, the new blood vessels associated with fibroblasts and macrophages replace the fibrin matrix with granulation tissue, forming a new substrate for keratinocyte migration[34] (Figure 1).

The resolution of the inflammatory response is mainly mediated by families of local-activity mediators that are biosynthesized from the essential fatty acids eicosapentaenoic acid and docosahexaenoic acid. These resolution mediators are termed resolvins, maresins and protectins[35]. Inflammation resolution is also mediated by lipoxins that are generated through platelet-leukocyte interactions[36] (Figure 3). It has been also proposed that regulatory T cells (Treg cells) have evolved to provide a complementary immunological arm to a physiological tissue-protecting mechanism driven by low oxygen tension, *i.e.*, hypoxia, in the inflamed tissues. The hypoxia-adenosinergic pathways might govern the production of immunosuppressive molecules that have already been implicated in the activities of Treg cells[37]. In this way, Treg cells could exert their suppressive function with local downregulation of immune response, inducing "*immunodormancy*" and protecting tissues from collateral tissue damage, thus improving healing[37]. The progressive resolution of inflammation favors wound re-epithelization. Fibroblasts can also contribute to the resolution of inflammation by withdrawing survival signals and normalizing chemokine gradients, thereby allowing infiltrating leukocytes to undergo apoptosis or leave the tissues through the draining lymphatics[38]. Remodeling begins two to three weeks after injury and lasts for a year or more. Most of the endothelial cells, macrophages and myofibroblasts, undergo apoptosis, leaving a mass that contains few cells and consists mostly of collagen and other extracellular-matrix proteins[34]. However, the prognosis of extensive and deep wounds is not entirely satisfactory because of

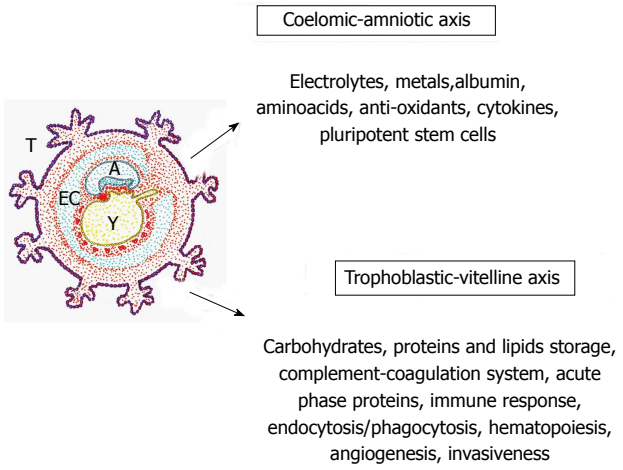

Figure 4 Hypothesized functions by ontogenic recapitulation in the traumatized tissue. These functions could be similar to the extra-embryonic coelomic-amniotic and trophoblastic-vitelline functions during early embryonic development. The extra-embryonic coelom or exocoelomic cavity surrounds the blastocyst, which is composed of the amnion and the primary yolk sac. EC: Exocoelomic cavity; A: Amnion; T: Trophoblast; Y: Yolk sac or vitellum.

scar formation and loss of normal function and skin appendages. Therefore, reducing the formation of scars and re-establishing the normal anatomy and function of the skin and its appendages have become the aim of regenerative medical research[39,40].

WOUND HEALING REPAIR USING EMBRYONIC MECHANISMS

Inflammation, whether acute or chronic, produces tissue remodeling[9]. In this way, it has been proposed that the inflammatory response has features in common with tissue development, which requires involution of pre-existing tissue elements[15,16]. The ability of the tissues to involute or dedifferentiate could represent a return to early stages of development[41]. Particularly, involution or dedifferentiation could form an effective defense mechanism to escape death after injury. Thus, this mechanism could make retracing an ancient, efficient and well-known route possible for repairing the injured tissue, just like the initial phases of embryonic development[2,41]. The correlation that can be established between the embryonic and the inflammatory events suggests that the results obtained from research into both great fields of knowledge would favor each other and promote their development[41].

In the adult body, many pathways that play an essential role during embryological development are inactivated later in life, although some of them may be transiently expressed during the adult repair process[41,42]. This ability of the tissues to involute or dedifferentiate could constitute an effective solution against any type of injury. Through dedifferentiation, tissues have the chance to reform and remodel themselves according to the new environmental situation imposed on them[10].

The fetus is uniquely capable of healing skin wounds without scar formation and provides a model of ideal tissue repair. Understanding the biology of this process may allow us to modulate wound healing in children and adults to become more fetal-like[43-45]. Tissue repair in the embryo and to a certain extent in adults too, appears to recapitulate those cell machineries used by embryos to undergo the natural tissue movements of morphogenesis, such as gastrulation and neural tube closure[41,46]. One key difference between embryonic and adult repair, which may explain why one heals perfectly and the other scars, is the presence of an inflammatory response at sites of adult repair while there is none in the embryo. However, total knockdown of inflammation is clearly not going to be an optimal treatment for post-natal scarring[46]. The infiltration of platelets, mast cells, neutrophils and macrophages which characterizes the early postnatal wound is greatly diminished in fetal wounds[43,47]. However, fetal wound healing is additionally characterized by a distinct extracellular matrix, anti-inflammatory and growth factor profile and a more important role for stem cells[5,6]. If so, we could hypothesize that to promote adult wound repair by regeneration, current therapies need to be attempted to recapitulate singular aspects of the fetal regenerative phenotype[5]. The evidence suggests that there may be an early critical window in postnatal wound healing that may be amenable to manipulation so as to provide a permissive environment for scarless wound healing to proceed[5].

In this way, the early post-traumatic inflammatory response could recapitulate ontogeny by re-expressing two hypothetical extra-embryonic trophic axes, that is amniotic and yolk sac or vitelline in the interstitial space of the injured tissue[41] (Figure 4). Likewise, the body could be repaired according to embryonic biochemical patterns through the expression of extra-embryonic functions. If so, the early inflammatory steps could represent the postnatal debut of ancestral biochemical mechanisms that were used for normal embryonic development. The re-expression of these ancient mechanisms is perhaps hard to recognize because they are anachronistic during postnatal life and are established in a different environmental medium[41,48] (Table 1).

After fertilization, the first stage of embryogenesis is the zygote, which undergoes cleavage by mitosis. When the morula stage is reached, the embryo establishes polarity. The cells bind tightly to each other, forming a compact sphere with two cell layers. The outer most layer becomes the trophoblast, giving rise to the placenta, and the inner cells become the inner cell mass, giving rise to the embryo and the remaining structures, including the amnion, yolk sac and allantoids[49] (Figure 4). The molecular and cellular contributions of the extra-embryonic tissues surrounding the fetus, namely the exocoelomic cavity, the amnion, the trophoblast and the yolk sac, to the interstitial space located between them, the mesoderm, are essential for organogenesis. In fact, the intra-embryonic mesoderm generated during gastrulation may represent the internalization of the functions that charac-

Table 1 Upregulation of extraembryonic phenotypes that could be involved in the different types of the wound healing reaction

Phenotypes	Embryonic functions	Phases of the inflammatory response	Phases of the wound healing reaction	
Extraembryonic phenotypes	Coelomic-amniotic axis	Nervous phase	Neurogenic systemic response	Stress response - Biogenic amines release Sensitive and motor alterations Ischemia-reperfusion - Local oxidative and nitrosative stress Hydroelectrolytic alterations - Edema Inflammation blood cells - Coagulation
	Trophoblastic-vitelline axis	Immune phase	Bone-marrow related response	Enzymatic stress Corticosuprarenal hormones - Local storage Hematopoietic stem cells Mesenchymal stem cells Endothelial progenitor cells
Embryonic phenotypes	Gastrulation	Angiogenic phase	Remodeling response	Myofibroblasts Angiogenesis Endothelial egg Re-epithelization Fibrosis

terize these extra-embryonic functions[50].

The hypothetical recapitulation of these initial phases of the embryonic development during the early surgical inflammatory response would imply the expression of functions similar to the extra-embryonic structures. Accordingly, the phenotype that could be adopted by the inflamed interstitium may induce the accumulation of fluid with similar characteristics to coelomic fluid. In essence, interstitial edema with high levels of proteins, in particular albumin, as well as electrolytes, metals, amino acids, antioxidants, cytokines and cholesterol-derived hormone, would be produced in the inflammatory exudates[51,52]. Amnion-derived multipotent progenitor cells also secrete a unique combination of cytokines and growth factors called the "*amnion-derived cellular cytokine solution*" which establishes a connection between mesenchymal and epithelial cells during embryo development[53]. In this sense, the amniotic fluid surrounding the fetus may therefore be an extension of the extracellular space of the fetal tissues[54]. The amniotic-like phenotype could also offer the stem cell a hypoxic and hydrated interstitial axis with cytokines and growth factors, favoring not only nutrition by diffusion, but also transport, excretion and bacteriostatic and anti-inflammatory protection[54,55] (Figure 4).

The wall of the secondary yolk sac is formed by an external mesothelial layer, a vascular mesenchyme, with blood islands that promote the development of hematopoiesis and angiogenesis[56] and an endodermal layer facing the yolk sac cavity[53]. The mesothelial and endodermal layers have absorptive functions and are active in endocytosis/digestion[56,57]. In addition, the endodermal layer is the source of several proteins including acute phase proteins[58]. A major function of the yolk sac is carbohydrate, protein and lipid accumulation for embryo nutrition (*vitellum*)[57]. In addition, through the synthesis and release of acute phase proteins, this extra-embryonic phenotype reduces oxidative, nitrosative and enzymatic stress, activates the complement-coagulation system, regulates the lipid metabolism and favors phagocytosis[59]. During trophoblast differentiation, trophoblastic cells also exhibit intense phagocytic activity leading to events as diverse as engulfment and destruction of extracellular material and the production of inflammatory mediators that may modulate both the immune and trophoblast invasiveness[60,61] (Figure 4).

The molecular and cellular contribution made by the above-mentioned extra-embryonic membranes, *i.e.,* exocoelomic cavity, amnion, yolk sac and trophoblast to the intra-embryonic mesoderm, could be essential for embryo development and organogenesis. Moreover, these primitive extra-embryonic structures can be internalized by the embryo at early development stages[50]. Consequently, the hypothesized re-expression of these extra-embryonic functions after injury during postnatal life could be a key process needed to repair the injured organism[2,41]. If so, the recapitulation of extra-embryonic functions through the organism could be internalized into the injured interstitium, thus inducing a process similar to the early embryonic process for tissue repair by regeneration and/or fibrosis.

INFLAMMATORY ENDOTHELIAL EGG

It could be proposed that recapitulation of extra-embryonic functions during wound repair is made up through the activation of two functional axes, namely: the coelomic-amniotic axis and the trophoblastic-vitelline axis. Both axes would polarize in the interstitium of the wounded tissue, thus promoting the development of a new tissue (Figure 4).

In surgical-related inflammation, the interstitium is surrounded by an inflamed heterogeneous endothelium. Thus, this inflammatory endothelium would get cellular and molecular mediators through the post-capillary venule endothelium, the high endothelial venule endothelium in the lymph nodes and, to a lesser degree, through the capillary endothelium. Ultimately, the lymphatic endothelium has a basic excretory function. The complex made up by this inflamed heterogeneous endothelium and the interstitial space of the injured tissue surrounded by it

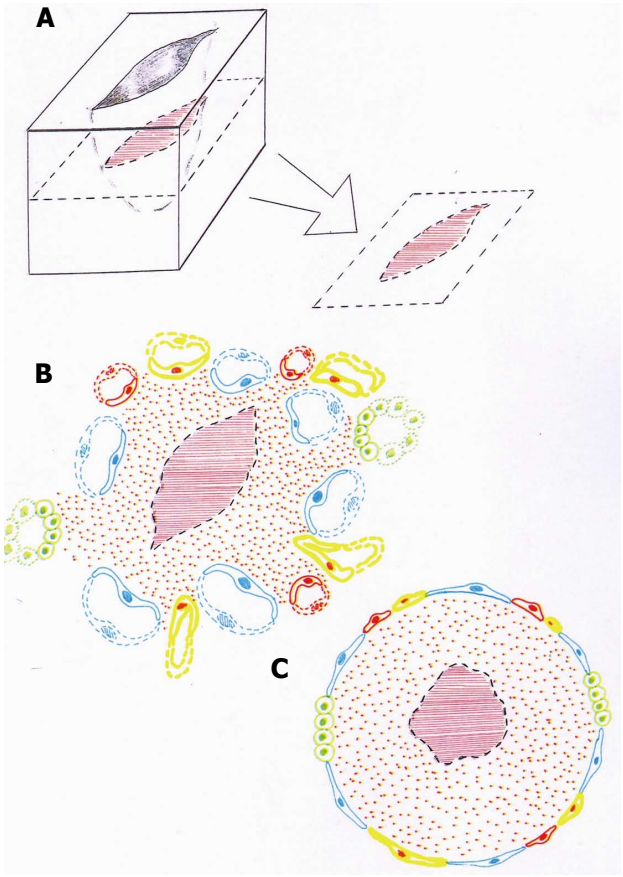

Figure 5 Figurative representation of a skin wound. The wound (A) is surrounded by different types of inflammatory venous, arterial and lymphatic endothelia (B). This heterogeneous inflammatory endothelium could be represented like a sheath of the inflamed interstitium that surrounds in turn the wound or broken tissue (C).

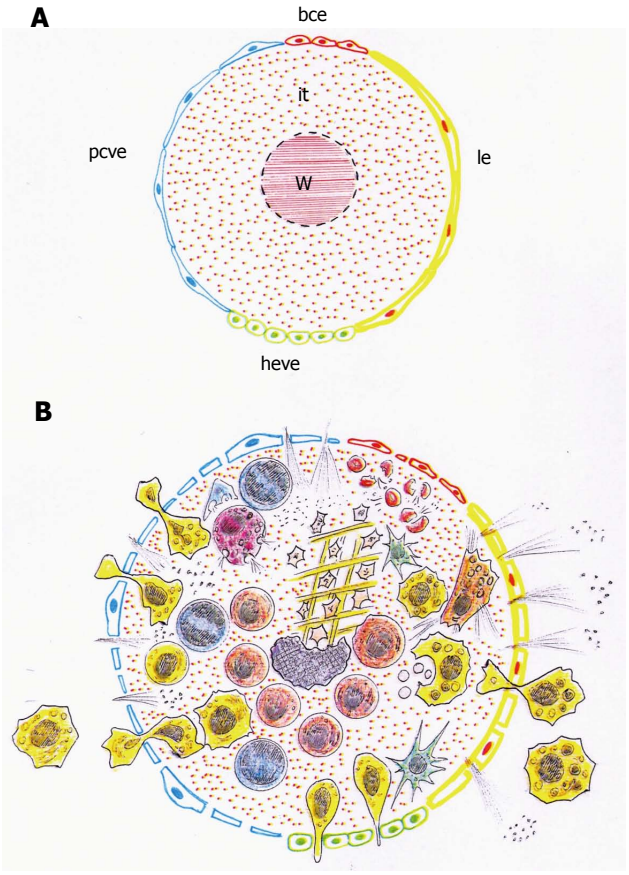

Figure 6 Schematic representations of the heterogeneous endothelium that surrounds the wounded tissue. A: The endothelium that cover the wound (W) and the damaged interstitium (it) are made up by the post-capillary venous endothelium (pcve), the high endothelial venular endothelium (heve), the lymphatic endothelium (le) and the blood capillary endothelium (bce); B: The inflammatory response is produced into the injured interstitium. The inflammatory mediators, molecules and cells, invade this interstitial space crossing through a sheath of heterogeneous endothelia.

has been compared with an "*endothelial egg*"[62] (Figures 5 and 6). Thus, in the interior of this heterogeneous endothelial sheath, the successive evolutive phases of wound repair with interstitial edema, activation of the lymphatic circulation and a hypoxic environment that could be an ideal stem cell niche, can be represented. Then, hemostasis by the formation of a platelet-fibrin clot occurs. After that, neutrophils, monocytes and lymphocytes are recruited and finally, new tissue is formed by regeneration, *i.e.*, keratinocytes and granulation tissue, *i.e.*, fibroblasts and endothelial cells, which form a substrate to complete the wound repair by fibrosis[12,14,30,34] (Figures 6 and 7).

However, cutaneous wound healing is not only a local process, but also a complex process involving systemic inflammatory alterations related to the stress response[2,62]. The magnitude of this systemic response may reflect the demands of the "*endothelial egg*" required for wound repair (Figure 7). In this sense, we have been trying to establish similarities between the complex pathophysiological mechanisms developed in wound healing and the pluripotential extra-embryonic pathways during embryonic development[2,10,41,62]. In this way, the recapitulation of coelomic-amniotic and trophoblast-vitelline functions is selectively integrated into the injured area. The recapitulation of the extra-embryonic coelomic and amniotic functions could be represented by initially activating the systemic neurogenic axis, while the latter recapitulation of the trophoblast and yolk sac functions would be carried out by activating the systemic bone-marrow axis (Figure 7).

RECAPITULATED COELOMIC-AMNIOTIC FUNCTIONS: A NEUROGENIC SYSTEMIC RESPONSE

The pathological neuromuscular response secondary to a wound induces sensory changes (stress, inflammatory pain, analgesia) and motor alterations (fight-to-flight and withdrawal reflexes, tachycardia and vasoconstriction-vasodilation). This upregulated extra-embryonic phenotype would induce a sudden and early neurogenic response with systemic cardiovascular, hemodynamic and hydroelectrolytic alterations[2,62]. Systemic and local ischemia-reperfusion produce sudden hydroelectrolytic changes associated with abnormal ion transport[63]. In this early response, cells that produce substances for export first

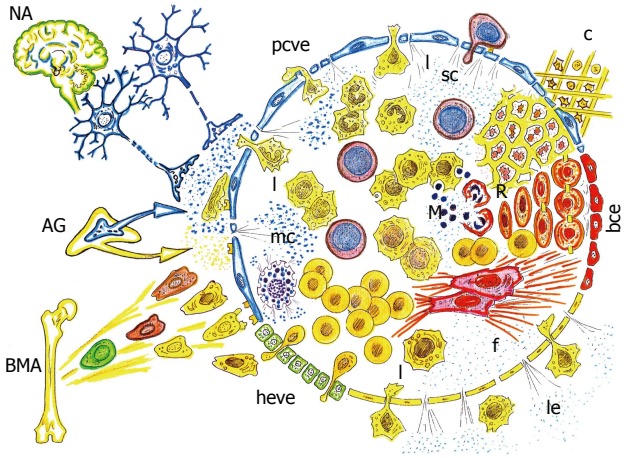

Figure 7 Neurogenic and bone marrow-related axes coupled in the inflamed endothelial egg, after wound. The upregulated extra-embryonic functions, *i.e.*, coelomic-amniotic or neurogenic, and trophoblastic-vitelline or bone marrow-related, are focused in the endothelial inflammatory egg, favoring the induction of a gastrulation-like phenotype, which evolves towards re-epithelization and fibrosis (scar) in post-natal life. NA: Neurogenic axis; AG: Adrenal gland; BMA: Bone marrow-related Axis; c: Coagulation; sc: Stem cell; mc: Mast cell. R: Regeneration; f: Fibrosis; l: Leukocytes; M: Microbiome. ve: Post-capillary venous endothelium; heve: High endothelial venular endothelium; le: Lymphatic endothelium; bce: Blood capillary endotelium; pcve: Psot capillary venous endothelium.

synthesize and then store large amounts of molecules, such as biogenic amines and neuropeptides in secretory vesicles ready for rapid release[64]. In this early neurogenic response, the activation of the hypothalamic-pituitary-adrenocortical, sympathetic-adrenal medullary and renin-angiotensin-aldosterone axes occur, with the release of catecholamines, glucocorticoids and mineralocorticoids. Consequently, selective accumulation of these mediators in the "*endothelial inflammatory egg*" is produced because endothelial permeability is increased, especially in postcapillary venules[2,62] (Figure 7).

RECAPITULATED TROPHOBLASTIC-VITELLINE FUNCTIONS: A BONE-MARROW-RELATED RESPONSE

The inflammatory bone marrow-related response induced by wounds could be considered both a key and complementary arm of the systemic response to injury. The inflammatory activation of the bone marrow stem cell niche indicates the stimulation of hematopoietic stem cells and mesenchymal stem cells, both which are multipotent stem cells[65-67]. Hematopoietic stem cells are the progenitors of all blood and immune cells. Macrophages generated from hematopoietic stem cells are the dominant phagocytes at wound-healing sites. Profibrotic macrophages, in particular, are intimately involved in wound healing through the production of mediators that directly activate fibroblasts, including transforming growth factor-beta (TGF-β), PDGF and IGF-1[28]. Nevertheless, although macrophages are required for the initiation and maintenance of fibrosis, they are also involved in its suppression, resolution and reversal[28,68]. Therefore, macrophage activation is best considered as a continuous spectrum of phenotypic characteristics[69]. In this context, circulating endothelial cells have also proved to be an important marker of vascular remodeling associated with wound healing. Angiogenesis is needed during embryonic development and plays important roles in wound healing and tissue ischemia throughout postnatal life[62]. Although the major physiological role of circulating endothelial progenitor cells is to maintain vascular integrity, they can also participate in revascularization of ischemic wounded tissues[70].

Furthermore, the upregulated trophoblastic-vitelline phenotype could mediate the inflammatory response through a lipid metabolic switch linked to steroid and acute phase response protein synthesis, respectively[2]. This slower response would therefore be developed by steroidogenic cells that store very little steroid hormones, in which case a rapid steroidogenic response would require immediate synthesis of new steroids, such as cortisol. The increase of the acute phase protein synthesis, *i.e.,* innate immunity, by the gut-liver axis is linked with the acute phase response and follows the upregulation of pro-inflammatory cytokines and chemokines[2,41,62].

COUPLING THE RECAPITULATED EXTRA-EMBRYONIC AXES IN THE "*INFLAMMATORY ENDOTHELIAL EGG*"

The systemic recapitulated extra-embryonic axes, *i.e.*, coelomic-amniotic and trophoblastic-vitelline, are focused and coupled in the endothelial inflammatory egg. This interstitial integration of both pathological axes, *i.e.*, neurogenic and bone-marrow-related in the wounded tissue, could finally induce a gastrulation-like process[41] (Figures 6 and 7). Gastrulation, which involves the "*de novo*" formation of reparative tissue, is based on the recapitulation of the intra-embryonic mesenchyme formation process[41]. In essence, the integration of both extraembryonic-related phenotypes coelomic-amniotic and trophoblastic-vitelline by the multipotent mesenchymal stem/stromal cells[67,71,72] would support the functional and metabolic heterogeneity needed for successively modulating their injured microenvironment during embryo development[50]. Therefore, the interaction of extra-embryonic functional axes recapitulated after injury in the interstitium of the damaged tissue allows for the recapitulation of the mechanisms characteristic of gastrulation, subsequently forming a mesenchyme in the endothelial inflammatory egg similar to that present in the early development phases[2,62].

Therefore, the early post-injury induction of extra-embryonic mechanisms that favors the beginning of the repair process[1] is undermined throughout the evolution of the wound healing reaction. In this way, the tissue that initiates its development inside the hypothesized endothe-

lial egg seems to suffer an immunological injury from the host organism. This reaction, similar to what takes place in organ transplantation, *i.e.*, host-versus-graft reaction, would explain the involution of the newly formed tissue until constructing, in the long term, the devitalized scar tissue. The study of those factors that induce this switch in the host organism, by which it gives up its gestating role and adopts a rejection attitude against already newly formed tissue, would explain why some authors consider that, in order to achieve tissue repair, inflammation is not needed[11].

CONCLUSION

In the current review, the wound healing reaction is considered a systemic inflammatory response made up by upregulated extra-embryonic functions, *i.e.*, coelomic-amniotic and trophoblastic-vitelline. The confluence and overlapping of these functions produce an injured tissue that would adopt an egg-like configuration that is one mainly made up of two structures: a round interstitial space surrounded by a heterogeneous endothelium. Therefore, cellular and molecular mediators from the extra-embryonic functions recapitulated by the injured organisms would induce a gastrulation-like process in this inflammatory endothelial egg from which tissue repair is produced either by regeneration and/or fibrosis.

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

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14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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