医学生物学 SCI 论文经典句子汇编

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Title

要求简练，精确

- Compassionate use of bevacizumab (Avastin) in children and young adults with refractory or recurrent solid tumors.
- Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy.
- Proteomics Approaches to the Systems Biology of Cardiovascular Diseases
- Pre- and post-natal treatment of hemophagocytic lymphohistiocytosis.
- Lack of early bevacizumab-related skeletal radiographic changes in children with neuroblastoma.
- Interleukin-4 activates androgen receptor through CBP/p300
- Trisomy 8 in an allogeneic stem cell transplant recipient representative of a donor-derived constitutional abnormality.
- Disruption of diacylglycerol metabolism impairs the induction of T cell anergy
- T cell anergy is reversed by active Ras and is regulated by diacylglycerol kinase-α
- High-dose conformal RT improves tumor control in patients with prostate cancer
- Vitamin D concentration does not affect the risk of prostate cancer
- Liver resection with salvage transplantation for hepatocellular carcinoma
- The impact of histopathologic diagnosis on the proper management of testis neoplasms
- Prostate stem cell antigen is associated with diffuse-type gastric cancer
- Multiple myeloma: high-risk immunophenotypes identified
- Increased c-kit expression predicts poor outcome in acute myeloid leukemia
- Global Analysis of the Meiotic Crossover Landscape
- Serum Response Factor Is Required for Sprouting Angiogenesis and Vascular Integrity
- Integrin Trafficking Regulated by Rab21 Is Necessary for Cytokinesis
- Reduced Translocation of Nascent Prion Protein During ER Stress Contributes to Neurodegeneration
- Effects of oral niacin on endothelial dysfunction in patients with coronary artery disease: Results of the randomized, double-blind, placebo-controlled INEF study.
- Global experiences with vardenafil in men with erectile dysfunction and underlying conditions.

- Noninvasive cardiac imaging: implications for risk assessment in adolescents and young adults.
- Transforming growth factor beta1 T29C gene polymorphism and hypertension: Relationship with cardiovascular and renal damage.
- A comparison of hormone therapies on the urinary excretion of prostacyclin and thromboxane A2.
- Repair of an infected aortic aneurysm using an aortic allograft and a venous autograft: Report of a case.
- Circulating Leptin and Stress-induced Cardiovascular Activity in Humans.
- Effects of aspirin dose on ischaemic events and bleeding after percutaneous coronary intervention: insights from the PCI-CURE study.
- Long-term cardiovascular outcomes following ischemic heart disease in patients with and without peripheral vascular disease.
- Reduced renal function and sleep-disordered breathing in community-dwelling elderly men.
- Intracoronary pharmacotherapy in the management of coronary microvascular dysfunction.
- Inhibition of platelet aggregation by combined therapy with aspirin and cilostazol after off-pump coronary artery bypass surgery.
- Inhibition of CCR2 Ameliorates Insulin Resistance and Hepatic Steatosis in db/db Mice

Abstract 要求简洁，连贯

- The acquisition of metastatic ability by tumor cells is considered a late event in the evolution of malignant tumors. We report that untransformed mouse mammary cells that have been engineered to express the inducible oncogenic transgenes MYC and $Kras^{D12}$, or polyoma middle T, and introduced into the systemic circulation of a mouse can bypass transformation at the primary site and develop into metastatic pulmonary lesions upon immediate or delayed oncogene induction. Therefore, previously untransformed mammary cells may establish residence in the lung once they have entered the bloodstream and may assume malignant growth upon oncogene activation. Mammary cells lacking oncogenic transgenes displayed a similar capacity for long-term residence in the lungs but did not form ectopic tumors.
- Almost two decades after *CFTR* was identified as the gene responsible for cystic fibrosis (CF), we still lack answers to many questions about the pathogenesis of the disease, and it remains incurable. Mice with a disrupted *CFTR* gene have greatly facilitated CF studies, but the mutant mice do not develop the characteristic manifestations of human CF, including abnormalities of the pancreas, lung, intestine, liver, and other organs. Because pigs share many anatomical and physiological features with humans, we generated pigs with a targeted disruption of both *CFTR* alleles. Newborn pigs lacking CFTR exhibited defective chloride transport and developed meconium ileus, exocrine pancreatic destruction, and focal biliary cirrhosis, replicating abnormalities seen in newborn humans

with CF. The pig model may provide opportunities to address persistent questions about CF pathogenesis and accelerate discovery of strategies for prevention and treatment.

- Variable lymphocyte receptors (VLRs) rather than antibodies play the primary role in recognition of antigens in the adaptive immune system of jawless vertebrates. Combinatorial assembly of leucine-rich repeat (LRR) gene segments achieves the required repertoire for antigen recognition. We have determined a crystal structure for a VLR-antigen complex, VLR RBC36 in complex with the H-antigen trisaccharide from human blood type O erythrocytes, at 1.67 angstrom resolution. RBC36 binds the H-trisaccharide on the concave surface of the LRR modules of the solenoid structure where three key hydrophilic residues, multiple van der Waals interactions, and the highly variable insert of the carboxyl-terminal LRR module determine antigen recognition and specificity. The concave surface assembled from the most highly variable regions of the LRRs, along with diversity in the sequence and length of the highly variable insert, can account for the recognition of diverse antigens by VLRs.
- A 51-year-old man with a diagnosis of myelodysplasia and non-Hodgkin's lymphoma underwent an unmatched allogenic bone marrow transplantation and was treated posttransplant with chronic immunosuppressive medication. Eight months following transplantation, he presented with progressive dysarthria, cognitive and visual decline. Evaluation included brain magnetic resonance (MR) imaging demonstrating multifocal areas of increased T2 and FLAIR (fluid attenuated inversion recovery) signals involving the left frontal, parietal, and occipital lobes. The MR lesions demonstrated diffuse increased signal on DWI (diffusion-weighted images) and normal to low signal on ADC (apparent diffusion coefficients). Contrast-enhanced T1 images were unremarkable. Lumbar puncture revealed a mild elevation in cerebrospinal fluid (CSF) protein. CSF PCR assay for viral DNA fragments were negative on two occasions. Serum serology for HIV was negative as well. A brain biopsy was subsequently performed. The clinical and neuroimaging differential diagnoses as well as neuropathologic correlation are presented.
- In vitro-generated mesenchymal stem cells (MSCs) initially attracted interest for their ability to undergo differentiation toward cells of different lineages.
- These results suggested that
- However, there are still obstacles in
- The major challenge for successful drug development is identifying delivery strategies that can be translated to the clinic.
- This review will discuss progress in developing and testing small RNAi-based drugs and potential obstacles.
- This review highlights what
- In addition, there are indications that
- Proper consideration of all of these issues will be necessary in
- These studies provide
- This paper presents the potential applications and the hurdles facing anti-HCV siRNA drugs.
- The present review provides insight into the feasible therapeutic strategies of siRNA technology, and its potential for silencing genes associated with HCV disease.

- A basic problem in the design of xx is presented by the choice of a xx rate for the measurement of experimental variables.
- This paper examines a new measure of xx in xx based on fuzzy mathematics which overcomes the difficulties found in other xx measures.
- This paper describes a system for the analysis of the xx.
- The method involves the construction of xx from fuzzy relations.
- The procedure is useful in analyzing how groups reach a decision.
- The technique used is to employ a newly developed and versatile xx algorithms.
- The usefulness of xx is also considered.
- A brief methodology used in xx is discussed.
- The analysis is useful in xx and xx problem.
- A model is developed for a xx analysis using fuzzy matrices.
- Algorithms to combine these estimates and produce a xx are presented and justified.
- The use of the method is discussed and an example is given.
- Results of an experimental applications of this xx analysis procedure are given to illustrate the proposed technique.
- This paper analyses problems in
- This paper outlines the functions carried out by ...
- This paper includes an illustration of the ...
- This paper provides an overview and information useful for approaching
- Emphasis is placed on the construction of a criterion function by which the xx in achieving a hierarchical system of objectives are evaluated.
- The main emphasis is placed on the problem of xx
- Our proposed model is verified through experimental study.
- The experimental results reveal interesting examples of fuzzy phases of : xx,xx
- The compatibility of a project in terms of cost, and xx are likewise represented by linguistic variables.
- A didactic example is included to illustrate the computational procedure

Introduction 引证核心文献，提出假设，指出文章的核心观点

Beginning

- Over the course of the past 30 years, .. has emerged form intuitive
- We evaluated 508 participants who
- Acute kidney injury (AKI) is associated with an increased incidence of respiratory failure requiring mechanical ventilation, which greatly increases mortality
- The cause of respiratory failure in patients with AKI is incompletely understood
- However, lung injury also occurs after ischemia–reperfusion injury of other organs such as the liver, gut, and hind limb
- We have demonstrated previously that
- Given this background, we hypothesized that
- we demonstrate that
- Technological revolutions have recently hit the industrial world
- The advent of ... systems for has had a significant impact on the

- The development of ... is explored
- The concept of xx was investigated quite intensively in recent years
- There has been a turning point in ... methodology in accordance with the advent of ...
- A major concern in ... today is to continue to improve...
- It has become increasingly clear that
- In this paper, we focus on the need for
- This paper proceeds as follow.
- The structure of the paper is as follows.

Our study

- In this paper, we shall first briefly introduce…
- To begin with we will provide a brief background on the
- This will be followed by a description of the xx of the problem and a detailed presentation of how the required membership functions are defined.
- Details on xx and xx are discussed in later sections.
- Polyphenolic compounds are vasodilators and help to lower the risk of cardiovascular diseases.
- Taken together, our novel findings suggest that the EDR induced by the strawberry extract was mediated by activation of the PI3 kinase/Akt signaling pathway, resulting in phosphorylation of eNOS.

Objective / Goal / Purpose

- The purpose of the inference engine can be outlined as follows:
- The ultimate goal of the xx system is to allow the non;experts to utilize the existing knowledge in the area of manual handling of loads, and to provide intelligent, computer;aided instruction for xxx.
- The paper concerns the development of a xx
- The scope of this research lies in
- The main theme of the paper is the application of rule;based decision making.
- These objectives are to be met with such thoroughness and confidence as to permit ...
- The objectives of the ... operations study are as follows:
- The primary purpose/consideration/objective of
- The ultimate goal of this concept is to provide
- The main objective of such a ... system is to
- The aim of this paper is to provide methods to construct such probability distribution.
- In order to achieve these objectives, an xx must meet the following requirements:
- In order to take advantage of their similarity
- more research is still required before final goal of ... can be completed
- In this trial, the objective is to generate...
- for the sake of concentrating on ... research issues
- A major goal of this report is to extend the utilization of a recently developed procedure for the xx.
- For an illustrative purpose, four well;known OR problems are studied in presence of fuzzy data: xx.

- This illustration points out the need to specify
- Recent studies have further defined the role of SBP-2 in promoting UGA read-through,
- This concept has been further validated with the discovery of patients with impaired deiodinase activity due to a mutation in SBP-2
- The ultimate goal is both descriptive and prescriptive.
- A wealth of information is to be found in the statistics literature, for example, regarding xx
- This review will focus on the most recent progress achieved in this field, particularly the cellular and molecular aspects of local control of thyroid hormone signaling provided by deiodinases.
- A considerable amount of research has been done .. during the last decade
- A great number of studies report on the treatment of uncertainties associated with xx.
- There is considerable amount of literature on planning
- However, these studies do not provide much attention to undertainty in xx.
- Since then, the subject has been extensively explored and it is still under investigation as well in methodological aspects as in concrete applications.
- Many research studies have been carried out on this topic.
- Problem of xx draw recently more and more attention of system analysis.
- Attempts to resolve this dilemma have resulted in the development of
- Many complex processes unfortunately, do not yield to this design procedure and have, therefore, not yet been automated.
- Most of the methods developed so far are deterministic and /or probabilistic in nature.
- The central issue in all these studies is to
- The problem of xx has been studied by other investigators, however, these studies have been based upon classical statistical approaches.
- Applied ... techniques to
- Characterized the ... system as
- Developed an algorithm to
- Developed a system called ... which
- Uses an iterative algorithm to deduce
- Emphasized the need to
- Identifies six key issues surrounding high technology
- A comprehensive study of the .. has been undertaken
- Much work has been reported recently in these filed
- Proposed
- Presented
- State that
- Point out that the problem of
- Described
- Illustrated
- Indicated
- Has shown / showed
- Address

- Highlights
- A study on ...was done / developed by []
- Previous work, such as [] and [], deal only with
- The approach taken by [] is
- The system developed by [] consists
- A paper relevant to this research was published by []
- []'s model requires consideration of ..
- []' model draws attention to evolution in human development
- []'s model focuses on...
- Little research has been conducted in applying ... to
- The published information that is relevant to this research...
- This study further shows that
- Their work is based on the principle of
- More history of ... can be found in xx et al. [1979].
- Studies have been completed to established
- The ...studies indicated that
- Though application of xx in the filed of xx has proliferated in recent years, effort in analyzing xx, especially xx, is lacking.

提出 Problem / Issue / Question 或假设

- Unfortunately, real-world engineering problems such as manufacturing planning do not fit well with this narrowly defined model. They tend to span broad activities and require consideration of multiple aspects.
- Remedy / solve / alleviate these problems
- It has recently been reported that
- ... is a difficult problem, yet to be adequately resolved
- Two major problems have yet to be addressed
- An unanswered question
- This problem in essence involves using x to obtain a solution.
- An additional research issue to be tackled is ....
- Some important issues in developing a ... system are discussed
- The three prime issues can be summarized:
- The situation leads to the problem of how to determine the ...
- There have been many attempts to
- It is expected to be serious barrier to
- It offers a simple solution in a limited domain for a complex problem.
- There are several ways to get around this problem.
- As difficult as it seems to be, xx is by no means new.
- The problem is to recognize xx from a design representation.
- A xx problem can trace its roots to xx.
- xx [1987] used a heuristic approach to simplify the complexity of the problem.
- Several problems are associated with them.

- Although some progress has been made in this area, at least two major obstacles must be overcome before a fully automated system can be realized.
- Most problems in practice are complicated
- More problem surface here.
- Hamper effort toward a xx system
- In order to overcome the limitations due to incomplete and imprecise xx knowledge, a xx program has been developed, which bases its knowledge upon the statistical analysis of a sample population of xx
- The above difficulties are real challenges faced by researchers attempting to develop
- This type of mapping raises no controversy to the issue of membership function determination.
- However, attempts to quantify the xx have met both theoretical and empirical problems.
- It has become apparent that in order to apply this new methodological framework to real;world problems and data, we have to pay attention to the problems of xx and xx.

MATERIALS AND METHODS

Materials

- Chemicals were purchased from Sigma (St Louis, MO), if not stated otherwise. Experiments were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.
- CsA, EGF, PD98059, U0126, AG1478, Wortmannin, and LY294002 were from Calbiochem (San Diego, CA, USA). Anti-ERK1/2 and anti-Ras were from Transduction Laboratories (Franklin Lakes, NJ, USA). Anti-phospho Raf-1 (Ser^{259}), anti-phospho Raf-1 (Ser^{338}), anti-phospho PKB/Akt (Ser^{473}), anti-PKB, anti-phospho EGFR (Tyr^{1068}), anti-phospho ERK1/2 (Thr^{202}/Tyr^{204}), anti-PI3K 110α, anti-p53, and anti-phospho MEK1/2 ($Ser^{217/221}$) were from Cell Signalling (Danvers, MA, USA). Anti-MEK and anti-Raf-1 (C12) were from Santa Cruz (Santa cruz, CA, USA). Apigenin and all other reagents were from Sigma (Saint Louis, MO, USA).

Animal

- Eight- to ten-week-old male C57BL/6 mice (wild-type) and IL-6-deficient mice backcrossed over eight generations on a C57BL/6 background were used
- Mice were maintained on a standard diet and water was made freely available.
- All experiments were conducted with adherence to the NIH Guide for the Care and Use of Laboratory Animals.
- The animal protocol was approved by the Animal Care and Use Committee of the University of Colorado
- Three surgical procedures were performed as described previously:[5] (1) sham operation, (2) ischemic AKI, and (3) bilateral nephrectomy.
- The abdomen was closed in one layer.
- Sham surgery consisted of the same procedure except that clamps were not applied.

- For bilateral nephrectomy, renal pedicles were tied off with suture and then cut distally.
- The ureters were pinched off with forceps and the kidneys removed.
- Serum was collected as described previously.[5] Blood urea nitrogen and creatinine were measured using an autoanalyzer (Beckman Instruments, Fullerton, CA, USA).
- Serum IL-6 was measured by ELISA according to assay instructions (R&D Systems, Minneapolis, MN, USA).
- Five-micrometer sections of paraffin-embedded lung tissue were stained with hematoxylin and eosin using standard protocols. Neutrophils were counted on the basis of morphological criteria; at least 50 high-powered fields (×40) were counted per slide.
- Frozen lung was prepared for ELISA as described previously.[5] Supernatants were analyzed for protein content using a Bio-Rad DC protein assay kit (Hercules, CA, USA). KC and MIP-2 were determined by ELISA (R&D Systems, Minneapolis, MN, USA).
- One-fourth lung was used to determine MPO activity as described previously.
- Frozen lung was homogenized in radioimmunoprecipitation assay buffer with protease inhibitor; western blotting was performed as described previously.[49] Goat anti-murine ICAM-1 polyclonal antibody (R&D Systems, Minneapolis, MN, USA; 1:2000) or rat anti-murine VCAM-1 monoclonal antibody (R&D Systems; 1:1000) were used.
- A total of 20 μg anti-IL-6 antibody vs IgG control (eBioscience, San Diego, CA, USA) was administered to wild-type mice by tail vein injection 1 h before surgery, intraperitoneally at the time of clamp removal (ischemic AKI) or nephrectomy (bilateral nephrectomy) and intraperitoneally 1 h following surgery (60 μg total).

Experimental groups

- STZ-induced diabetic rats, a model of partial type I diabetes: SD rats received a single intraperitoneal injection of freshly prepared STZ (65 mg kg-1 body weight, dissolved in 100 mmol l-1 citric acid, pH 4.5), and confirmed 2 days later by PP blood glucose (>250 mg dl-1).
- CTR rats: Vehicle-injected SD rats after 2 to 7 days, 14 to 30 days, and 90 days served as CTR for the 2 and 7 days STZ, the 14 and 30 days STZ, and for the 90 days STZ, respectively.
- Insulin treatment in STZ: Glc was normalized in seven animals during 12–14 days of STZ by subcutaneous insulin implants (2U day-1; Lin Shin Canada, Ontario, Canada).

Cell Culture

- Immortalized cells from the convoluted portion of mouse kidney proximal tubule PKSV-PCT cells (PCT3 clone) were cultured in a medium A (DMEM/Ham's F12 (1:1, v/v), 20 mM HEPES, 2 mM L-glutamine, 12.5 mM D-glucose, 60 nM sodium selenite, 5 $\mu g\ ml^{-1}$ transferrin, 50 nM dexamethasone, 100 U ml^{-1} penicillin, and 100 $\mu g\ ml^{-1}$ streptomycin), supplemented with 2% fetal bovine serum, 5 $\mu g\ ml^{-1}$ insulin, 10 ng ml^{-1} EGF, and 1 nM triiodothyronine at 37°C in a 95:5 air/CO_2 water-saturated atmosphere. For all experiments, cells were seeded at 0.2 $\times 10^6$ cells/ml and after 24 h with complete medium cells were starved for 16 h in medium A supplemented with 0.1% fetal bovine

serum but not insulin, EGF, or triiodothyronine. CsA was dissolved in ethanol and all the pharmacological inhibitors were in DMSO. In all cases, controls were carried out with cells treated with the corresponding vehicle alone. After treatments, cells were washed twice with cold phosphate-buffered saline (PBS) and harvested with lysis buffer as in Llorens *et al*

Cell viability

- After treatments, PCT3 cells were harvested and washed twice with cold PBS, and the viable cells were counted with Trypan Blue Dye (Gibco-Life Technologies, Grand Island, NY, USA) in a Neubauer chamber. Living cells exclude the dye, whereas dead cells will take up the blue dye. For Hoechst staining, cells seeded in six-well dishes were washed twice with PBS and fixed for 15 min with 4% paraformaldehyde at room temperature. Then, cells were washed twice again with PBS and stained with Hoescht (5 μg ml-1 in PBS) for 5 min.

Western blots/ Immunoblot

- The protein content of cellular extracts was quantified by the Bradford assay.44 Twenty-five microgram of total cell extract protein was run on SDS-polyacrylamide gel electrophoresis gels, transferred onto polyvinylidene difluoride membranes, and incubated with the corresponding antibodies. The membranes were developed with the enhanced chemiluminescence method (Pierce, Rockford, IL, USA).
- Supernatants of growing or growth-arrested cells were centrifugated for 5 min at 10 000 *g*. The cells were lysed as described. The proteins from supernatant and cell lysates were concentrated using heparin sepharose. The heparin sepharose was washed four times with phosphate-buffered saline containing protease inhibitors, dissolved in phosphate-buffered saline/protease inhibitor and incubated with 500 μg protein over night at 4°C. The complexes were washed with phosphate-buffered saline/protease inhibitor and the proteins were eluated with 100 μl Laemmli buffer without bromophenol blue (10 min 95°C). A 30 μl probe was loaded in each lane and western blot analysis was performed as described, using a polyclonal antibody against CCN3 (K19M), which recognizes a C-terminal 19-aminoacid peptide of human CCN3. As a positive control, a supernatant from adrenocortical cell cultures, which are known to secrete CCN3, was used.
- Cells were lysed in 0.5% (volume/volume) Triton X-100 lysis buffer and immunoblot analysis was done as described[43]. Immunoprecipitation with anti-CrkL or control rabbit antiserum was done as described[44]. Antibodies to the following were used: phosphorylated Erk (910L; Cell Signaling); phosphorylated Jnk (V7932; Promega); Erk (13-6200; Zymed); Jnk1 (sc-474), H-Ras (sc-35), C3G (sc-869), CrkL (sc-319), RasGRP1 (sc-8430) and DGK-ζ (sc-8722; all from Santa Cruz Biotechnologies); and DGK-α (a gift from H. Kanoh, Sapporo Medical University, Sapporo, Japan). Images were scanned, followed by densitometry analysis with UN-SCAN-IT software (Silk Scientific).

- Purified splenic T cells were stimulated for various times with 5 μg/ml of anti-CD3ε (500A2; BD Pharmingen) and were lysed in 1% Nonidet P-40 lysis buffer (1% (volume/volume) Nonidet-40, 150 mM NaCl and 50 mM Tris, pH 7.4) with protease inhibitors. Proteins were resolved by SDS-PAGE and were transferred to a Trans-Blot Nitrocellulose membrane (Bio-Rad Laboratories); membranes were probed with antibodies specific to phosphorylated Erk (91015; Cell Signal Technology) and phospholipase C-γ1 (05-163; Upstate Biotechnology). Membranes were stripped and were reprobed for analysis of total Erk (SC-16982; Santa Cruz Biotechnology). Activated Ras in cell lysates was determined by glutathione *S*-transferase–Raf—Ras-binding domain precipitation assay as described

Immunofluorescence microscopy.

- Analysis of protein localization in 2C T cell–P815.B71 cell conjugates was done as described[29]. P815.B71 cells were labeled with CMAC (7-amino-4-chloromethylcoumarin) Cell-Tracker Blue (Molecular Probes) and were mixed with equal numbers of anergic or *in vitro*–primed 2C *Rag2*$^{-/-}$ T cells. After approximately 8 min, cells were fixed, were made permeable and were stained with anti-GRP1 and anti-talin (Santa Cruz Biotechnologies) and with species-specific secondary antibodies conjugated to fluorescein isothiocyanate or phycoerythrin, respectively. Samples were analyzed with a Zeiss Axiovert 100 microscope, and 15 conjugates were typically assigned scores. Slidebook software (Intelligent Imaging Innovations) was used for image capture and deconvolution analysis. ImageJ 1.36b software (US National Institutes of Health) was used for quantification of pixel intensity.

Measurement of ROS generation

- The assay is based on the incorporation of 2',7'-dichlorofluorescein diacetate into the cell. H2O2 and peroxidases are able to oxidize the cleaved DCFH to DCF, which is highly fluorescent at 530 nm. To measure CsA-induced ROS generation, cells were washed twice with PBS, and fresh medium containing 20 μM 2',7'-dichlorofluorescein diacetate was added to previously treated cells. After 30 min cells were washed again, tripsinized, and resuspended with cold PBS. Fluorescence was measure by flow cytometry on a FACScan flow cytometer.

Raf-1 activity

- Raf-1 immunoprecipitation and kinase assay were performed as described previously.45 Immunoprecipitated Raf was incubated for 30 min at 30°C with 0.8 mM ATP, 10 μg ml-1 GST-MEK, and 100 μg ml-1 GST-ERK2. An aliquot of the supernatant was used for ERK2 activity assays using 0.5 mg ml-1 myelin basic protein and 0.1 mM [γ-32P] ATP (400 c.p.m. pmol-1). After 15 min incubation at 30°C, 12 μl of 5 ×Laemmli loading buffer was added to the tubes and the mixture analyzed by SDS-polyacrylamide gel electrophoresis. Radiolabeled bands were quantified in a PhosphoImager.

Semiquantitative RT-PCR.

- Total RNA was isolated from freshly isolated thymocytes. Then, cDNA was prepared with the M-MuLV reverse transcriptase and random primers according to the manufacturer's recommendations (New England Biolabs). Semiquantitative PCR analysis of *Tcrb* VDJC (where 'C' is the constant region) and *Cd3e* cDNA was done as described[51]. [^{32}P]dCTP (GE Healthcare Life Science) was incorporated into PCR products for semiquantitative detection by autoradiography.

Real-time quantitative RT-PCR

- Total RNA was isolated from HMC or rat mesangial cells using the Invisorb Spin Cell-RNA Mini Kit (Invitek, Berlin, Germany) or from isolated glomeruli using the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA purity determination, cDNA synthesis, and RT-PCR were performed as described.16 Primer sequences are listed in Table 2. Glyceraldehyde-3-phosphate dehydrogenase cDNA amplification was used as an internal standard.
- Total RNA was isolated from the frozen kidneys as described by Chomczynski and Sacchi47 and quantified by a photometer. One microgram of the resulting RNA was used for reverse transcriptase (RT)-PCR. The cDNA was synthesized by MMLV reverse transcriptase (Superscript-Invitrogen, Carlsbad, CA, USA). For quantification of renin mRNA expression (sense: 5'-ATGAAGGGGGTGTCTGTGGGGTC-3', antisense: 5'-ATGCGGGGAGGGTGGGCACCTG-3'), real-time RT-PCR was performed using a Light Cycler Instrument (Roche Diagnostics Corp., Basel, Suisse) and the QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany), with GAPDH (sense: 5'-TTCATTGACCTCAACTACAT-3', antisense: 5'-GAGGGGCCATCCACAGTCTT-3') as a control. PCR was run for 30 cycles with 15 s per 95°C denaturation, 20 s/58°C annealing and 20 s/72°C elongation. To verify the accuracy of the amplicon, a melting curve analysis was done after amplification.Total renin mRNA content per kidney was calculated from the yield of RNA extracted from the whole kidneys times the renin mRNA estimate obtained from the defined amount of RNA used for RT-PCR real time measurement. For the RT-PCR real-time measurements, a pool of RNA from adult mouse kidneys was generated, which served as standard for all RT-PCR runs. Thus, all renin mRNA levels for the developing kidneys were estimated relative to the levels in adult kidneys.

In vitro anergy assay.

- Wild-type, Dgka-/- and Dgkz-/- splenocytes were stained with 5 μM CFSE, were stimulated for 72 h with anti-CD3 (1 μg/ml; 2C11) along with CTLA-4–Fc (5 μg/ml), were stained with allophycocyanin-conjugated anti-CD4 and were analyzed by flow cytometry. Cell division was assessed by CFSE dilution after gating on live CD4+ cells. Alternatively, cells were stimulated for 72 h and were pulsed with 1 μCi/well of [3H]thymidine for the final 8 h of stimulation, and proliferation was assessed by tritium incorporation with a scintillation counter. For restimulation analyses, cells were

prestimulated with anti-CD3 plus CTLA-4–Fc, then after 72 h, CD4+ cells were purified by negative selection (with fluorescein isothiocyanate–conjugated anti-CD8, anti-B220 (RA3-6B2; BD Pharmingen), anti-DX5 and anti-CD11b (M1/70; BD Pharmingen), followed by depletion with anti–fluorescein isothiocyanate magnetic beads) and were allowed to 'rest' overnight at 37 °C. Live cells were then counted by Trypan blue exclusion, and equivalent numbers of live cells were dropped onto monolayers of bone marrow–derived macrophages coated with anti-CD3 (1 μg/ml) and anti-CD28 (0.5 μg/ml). After 24 h, supernatants were collected and IL-2 was quantified by ELISA according to the manufacturer's protocol (R&D Systems).

Three-dimensional reconstruction

- Serial sections of kidney specimens were fixed and stained for renin and for αSMA as described above. Digitalization of the serial slices was performed using an AxioCam MRm camera (Zeiss, Jena, Germany) mounted on an Axiovert200M microscope (Zeiss) with fluorescence filters for renin and αSMA (TRITC: filter set 43: Cy2: filter set 38 HE; Zeiss). After acquisition, a stack of equal-sized images was built using the graphic tool ImageJ (Wayne Rasband, NIH, Bethesda, MD, USA). The equalized data were then imported into the Amira 4.1 visualization software (Mercury Computer Systems Inc., Chelmsford, MA, USA) on a Dell Precision 690 computer system (Dell, Frankfurt, Germany), and subsequently split into the renin and αSMA channels. After this step, the renin and αSMA channels were aligned. In the segmentation step, the αSMA and renin data sets served as a scaffold and were spanned manually or automatically using grayscale values. Matrixes, volume surfaces, and statistics were generated from these segments.

Restimulation assay after *in vivo* immunization.

- For analysis of T cell priming *in vivo*, $CD4^+$ T cells were collected from naive, primed or tolerized recipient mice on day 15 after immunization. Proliferative responses were measured by culture for 72 h of $CD4^+$ T cells (3×10^6 cells/ml) with irradiated (3,000 rads) APCs (10×10^6 cells/ml) and OVA(323–339). The number of KJ1-26^+ cells for each group of recipient mice was determined by flow cytometry and proliferation was normalized to the number of input KJ1-26^+ cells. Supernatants were collected from plates and cytokine concentrations were measured by ELISA.

Flow cytometry.

- For analysis of surface antigen expression, mAb to CD4 (JK1.5; eBioscience) and mAb KJ1-26 (KJ-126; Caltag) were used. For intracellular IL-2 staining, T cells were restimulated for 24 h *in vitro* with OVA(323–339) in the presence of APCs as described above. Brefeldin A (eBioscience) was added for the last 6 h of the culture. Cells were collected and were stained with allophycocyanin-conjugated mAb to CD4 and fluorescein isothiocyanate–conjugated mAb KJ1-26. Then, cells were fixed, were made permeable and were stained with antibody to IL-2 (clone JES6-5H4; eBioscience) according to the manufacturer's instructions.

- T_H1 cells transduced with adenovirus vector encoding GFP were analyzed with a FACScan (BD Biosciences). A total of 1×10^4 events were acquired, and data were analyzed with CellQuest software (BD Biosciences).
- Splenic and lymph node samples depleted of thymocytes and red blood cells were stained with fluorescence-conjugated anti-CD3 (2C11), anti-CD4 (GK15), anti-CD8 (53-6.7), anti-CD25 (7D4) and anti-CD44 (552407; all from BD Pharmingen). A three-color FACScan (Becton Dickinson) was used for flow cytometry, and data were analyzed with FlowJo 4.6 (TreeStar).
- A FACSCalibur (Becton Dickinson) was used for flow cytometry. Human cells from transplanted NOD-SCID mice were assessed with phycoerythrin–cyanin 5–conjugated anti–human CD45 and phycoerythrin-conjugated anti-CD19, anti-CD33, anti-CD36 and anti–glycophorin A (Becton Dickinson). EGFP fluorescence was detected with channel FL1 calibrated to the fluorescein isothiocyanate emission profile. During quadrant analysis, only fluorescence excluding more than 99% of isotypic control events was considered specific. Cell Quest Pro software (Becton Dickinson) and FlowJo (Tree Star) were used for data acquisition and analysis.

Mammalian expression plasmids and transfection.

- For generation of the plasmid expressing Smad3 shRNA, the following specific oligonucleotides were used: upper, 5'-GATCCACCTGAGTGAAGATGGAGATTCAAGAGATCTCCATCTTCACTCAGG TTTTTTTACGCGTG-3'; lower, 3'-AATTCACGCGTAAAAAAACCTGAGTGAAGATGGAGATCTCTTGAATCTCCA TCTTCACTCAGGTG-5'. These were cloned under control of the U6 promoter into the pSIREN-DNR-DsRed expression vector (Clontech, BD). Vector expressing shRNA specific for luciferase served as a control. Smad3-Tm was subcloned into the pIRES2-EGFP vector (Clontech, BD); empty vector served as a control. Purified DO11.10 or DO11.10p27A T cells were transfected with plasmids by nucleofection with the Amaxa nucleofection apparatus, according to the manufacturer's instructions (Mouse T Cell Nucleofector Kit Amaxa Biosytems). Purified T cells were suspended in nucleofector solution (3×10^6 cells/100 μl) and were mixed with 3 μg of plasmid. Samples were transferred into cuvettes, were transfected with nucleofector program X-01 and were then immediately transferred into 12-well plates and were cultured in nucleofector medium for 3 h. Then, cells were collected and counted and were immediately transferred into syngeneic recipient mice (3×10^6 cells per mouse). At 3 h after adoptive transfer, mice were given priming or tolerizing treatment *in vivo* according to the standard protocol described above. Lymphocytes were isolated from draining lymph nodes at day 5 of the treatment, $CD4^+$ T cells were purified and transfection efficiency was assessed by flow cytometry. The range of transfection efficiency was 69–75% (Supplementary Fig. 4 online). Smad3-knockdown and control-knockdown DO11.10 cells and DO11.10 cells transfected with Smad3-Tm and vector control were selected by cell sorting. The resulting $CD4^+$ T cells (2×10^6 cells/ml) were restimulated with OVA(323–339) (5 μg/ml) in the presence of irradiated APCs *in vitro*.

Luciferase assays.

- CAR IL-2–Luc T_H1 clones were transduced with vectors, were stimulated for 20 h and were resuspended in serum-free DMEM in luminometer cuvettes (BD Biosciences). An equal volume of Bright-Glo luciferase assay reagent (Promega) was added to each sample, followed by thorough mixing. After 2 min, samples were analyzed with a monolight 2010 Luminometer (BD Biosciences).

Analysis of cell divisions *in vivo*.

- Purified T cells from DO11.10 and DO11.10p27Δ mice (10×10^6 cells/ml) were labeled for 30 min at 37 °C with the intracellular fluorescent dye CFSE (5 μM 5(and 6)-carboxyfluorescein succunimidyl ester; Molecular Probes). Then, cells were washed twice with cold RPMI 1640 medium containing 10% FCS, were resuspended in PBS and were transferred intravenously into BALB/c mice (5×10^6 cells per mouse). Syngeneic hosts were left untreated (naive) or were treated with PBS followed by immunization with OVA(323–339) (primed) or with CTLA-4–Ig plus mAb to CD40L followed by immunization with OVA(323–339) as described above (tolerized). Then, 3 d later, lymphocytes were isolated from the draining lymph nodes of the BALB/c hosts. The number of cell divisions on CFSE-stained cells and the percentage of cells that had undergone a specific number of divisions were determined as described[43]. Cells were also stained with mAb KJ1-26 and CFSE analysis of KJ1-26$^+$ T cells was done by flow cytometry.

Adenovirus vectors.

- The cDNA encoding Ras61L was provided by F. Fitch (University of Chicago, Chicago, Illinois). The dominant negative Cbl construct was generated by RT-PCR with cDNA from T_H1 clones as a template and the following primers (upper case, restriction enzyme sequences; underlining, Myc tag sequence): 5'-GGGGTACCatggagcagaaactcatctctgaagaggatctggccggcaacgtgaagaaga-3' (forward) and 5'-ATAGTTTAGCGGCCGCtcaatcttgaggagttggtt cacataa-3' (reverse). The cDNA encoding DGK-α was a gift from M. Topham (University of Utah, Salt Lake City, Utah) and was used as a template to introduce an N-terminal Myc epitope tag by PCR. The sequences of all PCR products were confirmed before subcloning. Construction of recombinant adenovirus vectors was done with a two-cosmid system that has been described[42].

Adenoviral transduction of CAR T cells.

- T_H1 clones were purified from passage cultures by Ficoll-Hypaque centrifugation. Primary CAR 2C *Rag2*$^{-/-}$ CD8$^+$ T cells were isolated from splenocytes by negative selection with magnetic beads and antibody 'cocktails' (Stem Cell Technologies). CAR T_H1 cells were transduced with adenovirus vectors at high cell density (1×10^7 cells/ml) in DMEM containing 2% (volume/volume) FCS and were incubated for 1 h at 37 °C,

followed by an overnight 'rest' at 37 °C in DMEM containing 5% (volume/volume) FCS at low cell density (4 $\times 10^5$ cells/ml).

Lentivirus production and infection protocols.

- A third-generation lentiviral vector encoding EGFP expressed from the human phosphoglycerate kinase promoter was used as described[29, 33]. Cell populations were incubated overnight (about 16 h) in X-VIVO-10 medium (BioWhittaker) supplemented with 1% BSA (Stem Cell Technologies) and L-glutamine (Invitrogen) with viral supernatant (multiplicity of infection of 130–180). Viral concentrations of 1.0 $\times 10^8$ to 1.8 $\times 10^8$ viral particles/ml, 2.0 $\times 10^7$ to 4.4 $\times 10^7$ viral particles/ml and 0.9 $\times 10^8$ to 1.6 $\times 10^8$ viral particles/ml and cell concentrations of 0.7 $\times 10^6$ to 1.1 $\times 10^6$ cells/ml, 1.0 $\times 10^5$ to 2.5 $\times 10^5$ cells/ml and 0.7 $\times 10^6$ to 1.4 $\times 10^6$ cells/ml for $CD34^+CD38^{lo}$, $CD34^+CD38^-$ and Lin^- cord blood, respectively, were maintained. The efficiency of gene transfer was estimated by progenitor cell assay as described[33].

Apoptosis induction.

- Spontaneous apoptosis of PMNs was detected after 22 h of incubation in culture media. In some experiments, zVAD-fmk (10-50 μM), TNF (40 ng/ml), resolvin E1–methyl ester, aspirin-triggered lipoxin A_4 analog, PD1–methyl ester (10 nM) or TGF-β (10 ng/ml) was added. Vehicle treatment was 0.05% (volume/volume) ethanol. Peripheral blood T cells were activated by incubation for 3 d in 24-well plates coated with anti-CD3 (5 μg/ml; R&D Systems). Jurkat cells or activated peripheral blood T cells were incubated for 4–48 h with staurosporine (1–2 μM) or Fas ligand (0.05–5 ng/ml), after which cells were collected and used for flow cytometry or binding assays. In some experiments, zVAD-fmk (10–50 μM; R&D Systems) was added to cells 20 min before the addition of apoptosis-indu

Mice strains and genotyping.

- The 129/Sv *Rhoh*$^{-/-}$ mice were generated by Targeting Laboratory. The entire coding region of mouse *Rhoh* is in its third exon; the targeting vector was designed to replace the third exon of *Rhoh* with a neomycin-resistance cassette. The genotypes of *Rhoh* gene-targeted embryonic stem cells and transgenic mice were determined by Southern blot analysis of DNA digested with *Spe*I using a 5' *Rhoh* genomic DNA probe or by PCR analysis with primers. The 129/Sv *Rhoh*$^{-/-}$ mice were crossed with wild-type or p14 TCR (V α 2V β 8) transgenic mice on a C57BL/6J background to generate *Rhoh*$^{-/-}$ or $p14^{tg/+}$*Rhoh*$^{-/-}$ compound mice. Mice used were littermates derived from backcross generations with an N of more than 2. The 129S6/SvEvTac-*Rag2*$^{-/-}$ mice were purchased from Taconic Animal Models. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Cincinnati Children's Hospital Research Foundation (Cincinnati, Ohio).

Antibodies and GST fusion proteins.

- Fluorescence-conjugated monoclonal antibodies to the following mouse antigens were used for flow cytometry: CD4 (RM4-5), CD8α (53-6.7), CD25 (7D4), CD44 (IM7), TCR β-chain (H57-597), TCR γδ (GL3), TCR Vβ8, TCR Vβ5 (MR9-4), CD69 (H1.2F3), CD5 (53-7.3), Gr-1 (RB6-8C5), Mac-1 (M1-70), NK1.1 (PK136), Thy1.2 (53-2.1), CD45R–B220 (RA3-6B2), IgM (R6-60.2), BrdU (3D4) and Ter119 (Ly-76; all from Pharmingen). For immunoblot analyses, antibodies to the following were used: RhoH[9] (B4998), Zap70 phosphorylated at Y319 (17a), phosphorylated tyrosine (4G10) and Lat (45; Pharmingen); hemagglutinin (3F10; Roche); β-actin (AC-15; Sigma); CD3ζ (6B10.2; Santa Cruz Biotechnology); and Lat phosphorylated at Y191 (3584), Zap70 (99F2), phosphorylated p42-p44 (Thr202-Tyr204; 197G2) and p42-p44 (9102; Cell Signaling Technology). Primary antibodies were detected with the secondary antibodies horseradish peroxidase–conjugated goat anti-mouse (7076) or goat anti-rabbit (7074; both Cell Signaling Technology), or donkey anti-rat (sc-2956; Santa Cruz Biotechnology) using enhanced chemiluminescence detection (Cell Signaling Technology). GST fusion proteins were expressed in *Escherichia coli* BL21 (DE3) cells and were purified according to the manufacturer's recommendations (GE Healthcare Life Science). Purified GST fusion protein lysates were incubated for 1 h at 4 °C with glutathione–Sepharose 4B beads. Bead-bound GST fusion proteins were separated by SDS-PAGE and were quantified by Coomassie blue staining.

GST precipitation assay.

- Jurkat cells were lysed in GST lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 1% Nonidet-P40 and Complete Protease Inhibitors). Cell lysates were loaded onto columns of bead-bound GST fusion proteins. After columns were washed with GST lysis buffer containing 150 mM and 200 mM NaCl, bound proteins were eluted with GST lysis buffer containing 400 mM NaCl and SDS sample buffer, sequentially. Eluted proteins were detected by SDS-PAGE and Coomassie blue staining. Protein bands were identified with a Bruker Biflex III MALDI-TOF mass spectrometer (SpectroREADER; Sequenom) and Protein Mass Fingerprinting Mascot search (Matrix Science).

Subcellular fractionation.

- Cells were lysed by brief sonication on ice in a buffer of 250 mM sucrose, 20 mM Tris, pH 7.8, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM Na_3VO_4, 10 mM NaF and Complete Protease Inhibitors. Lysates were centrifuged to remove nuclei and debris (900*g* for 5 min at 4 °C). The P100 and S100 fractions were separated by centrifugation for 30 min at 100,000*g*. Membrane fractions were made soluble with MLB (Upstate) plus protease and phosphatase inhibitors. After centrifugation for additional 30 min at 100,000*g*, the detergent-insoluble cytoskeleton-containing fraction was resolved by 0.5% SDS-PAGE.

Assessment of Intracellular Calcium Concentration

- Jurkat cells cultured in calcium-free RPMI-1640 medium (Gibco BRL; number 22300-107) containing calcium-free 10% FBS were triggered by anti-Fas IgM. The treated cells were harvested at the indicated time points and incubated with Fluo-3-AM at a final concentration of 1 micromolar for 30 min at 37°C (Scoltock et al., 2000). The labeled cells (50,000 cells per treatment) were then analyzed by exciting the cells at 488 nm and examining the fluorescence emission of Fluo 3 at 530 nm with a FACS Scan, (Becton Dickinson). A one micromolar concentration of LPA was used as a positive control for Ca2+ induction. The data thus obtained was analyzed with the software Win MDI 2.8 and represented as contour plots.The effect of chelating intracellular calcium on translocation of annexin I was studied by culturing Jurkat cells in the presence of 10 micromolar BAPTA-AM, with or without the addition of anti-Fas IgM. Cells were harvested and fractionated as detailed above, and the S-100 fractions were assessed by immunoblotting for presence or absence of annexin I.

Mouse bone marrow transduction and transplantation.

- Retrovirus-mediated transduction of mouse bone marrow cells was done by published methods[49]. Prestimulated low-density bone marrow cells were infected with high-titer retrovirus supernatant on fibronectin-coated plates. Retrovirus supernatant was generated in the phoenix-gp cells with a mouse stem cell virus–based retroviral vector coexpressing EGFP and HA–RhoH as described[50]. $EGFP^+$ sorted cells were transplanted by intravenous injection into the sublethally irradiated (300 rads with a ^{137}Cs irradiator) *Rag2*$^{-/-}$ recipient mice. At 9 weeks after transplantation, thymus, peripheral blood, bone marrow, spleen and lymph nodes from each recipient mouse were collected for analysis of $EGFP^+$ chimerism and hematopoietic lineage by flow cytometry. Expression of HA–RhoH and HA–RhoHF73F83 in $EGFP^+$ sorted thymocytes of recipient mice was confirmed by immunoblot analysis.

Determination of renal morphology

- Kidney slices were postfixed in buffered 2% OsO4, dehydrated, and embedded in an Araldite-EM bed 812 mixture. Large sections were cut perpendicular to the renal capsule, containing cortex, and medulla. Thin (1 μm) sections were analyzed in a blinded manner for morphologic alterations, as previously detailed

Patient population

- Patients included in the study met the following criteria: (1) biopsy-proven IMN; (2) creatinine clearance $\geq$30 ml per min per 1.73 m2; and (3) persistent proteinuria >5 g per 24 h despite treatment with an HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitor, an ACEi, and/or ARB at maximal tolerated dose for at least 4 months. The Mayo Institutional Review Board and the Research Ethical Board, University Health Network, University of Toronto approved the study protocol. All patients gave written informed consent. Patients who had been on treatment with prednisone, cyclosporine, or

mycophenolic mofetil within the last 4 months or alkylating agents within the last 6 months were not included in the study. Patients with active infection, diabetes, or a secondary cause of MN (for example, hepatitis B, systemic lupus erythematosus (SLE), medications, malignancies) were also excluded.

Treatment

- At enrollment, a low-sodium (<4 g day-1) and low-protein (0.8 g per kg per day of high-quality protein) diet was recommended and patients were encouraged to maintain the same diet throughout the duration of the study. All patients received a similar conservative treatment regimen that included loop diuretics to control edema, an HMG-CoA reductase inhibitor, and an ACEi combined with an ARB if tolerated. β-Blockers and non-dihydropyridine calcium channel blockers, in that order, were added when required to control systolic blood pressures to <135 mm Hg in >75% of the readings. Patients who after a minimum of 4 months of conservative therapy and maximized Ang II blockade had proteinuria >5 g per 24 h received two i.v. infusions of rituximab at a dose of 1000 mg on days 1 and 15. To minimize infusion reactions, patients were premedicated with acetaminophen (1000 mg) and diphenhydramine hydrochloride (50 mg) orally. In addition, methylprednisolone (100 mg, i.v.) was given prior to the first rituximab infusion. B-cell depletion was defined as CD19+ count <5 cells per μl at any time and B-cell recovery was defined as CD19+ cell count >15 cells per μl. Patients treated with rituximab, who at month 6 had proteinuria >3 g per 24 h and in whom CD19+ B-cell counts had increased to >15 cells per μl, received a second course of rituximab treatment following the same protocol described above.

Follow-up

- In all patients, clinical and laboratory parameters including complete blood counts, electrolytes, serum albumin, B-cell flow cytometry for CD19+ B cells, serum immunoglobulin (IgG, IgM, IgA) levels, and a lipid panel were evaluated at study entry and at months 1, 3, 6, 9, and 12. Creatinine clearance and protein and creatinine excretion in the urine were assessed by performing two consecutive 24-h urine collections at each time point. Data were considered accurate when urinary creatinine excretion was consistent with a complete 24 h collection. The mean of the two measurements was considered for the analysis. The presence of HACAs was evaluated at baseline and at months 3, 6, 9, and 12.

Method / Approach / Study/ Technique

- A discussion is presented of a problem-solving system
- To improve the efficiency of the method, the following approach may be applied.
- In order to an investigation was made to find the causes of the
- Although large collections of rules and equations have been complied, none are generally accepted

- This approach will be explained and discussed thoroughly in the body of the report.
- This can be accomplished by
- This algorithm to compute the total cost can be described step by step as follows:
- The above preliminary analysis has provided important information
- Various methods have been proposed for selecting an optimum...
- These concepts have been applied to
- On the basis of the concept mentioned above,
- This can be achieved by
- In addition, tissues were stained for infiltrating lymphocytes (CD20 and CD3), and the amount of interstitial fibrosis was quantified by histomorphometry. Formalin-fixed, paraffin-embedded sections were cut onto coated glass slides. Following heat-induced antigen retrieval, sections were incubated at 20°C overnight with either anti-CD20 primary antibody or anti-CD3 primary antibody, both at 1:1000 dilution (Dako, Canada Inc, Mississauga, Ontario, Canada). After rinsing all sections, pretreatment with 3% hydrogen peroxide was performed to prevent endogenous peroxidase activation. Sections were incubated with a secondary rabbit anti-mouse antibody linked with avidin–biotin complex. Sections were counterstained with hematoxylin and examined by light microscopy.
- The HACA assay is a proprietary bridging enzyme-linked immunosorbent assay performed at Genentech Inc. that measures the antibody response to rituximab in human serum samples.
- In all patients, clinical and laboratory parameters including complete blood counts, electrolytes, serum albumin, B-cell flow cytometry for CD19+ B cells, serum immunoglobulin (IgG, IgM, IgA) levels, and a lipid panel were evaluated at study entry and at months 1, 3, 6, 9, and 12.
- This fact suggests that a new concept
- This was accomplished by taking ...
- The preparatory stage is very time consuming process.
- Test are performed for validity, completeness, and compatibility
- There is little hope of achieving successful ...
- There has been an increasing awareness of the potential of using most ..so far made have not taken this approach, with the exception of
- Only a few studies can be found.
- It is a very tedious process to go through
- It is only when .. has been completed that .. may be effected
- The entire interpretation process is conducted in one's head.
- These approaches are sometimes very tedious.
- Several techniques can be used
- A polynomial parametric model can be written as [the following]/[follows]:
- A xx model is constructed/formulated using xx.
- A xx model represents an xx by its xx.
- A process decision model captures the logic essential to
- From the equation above, xx is equal to the summation of xx times the ...

- The validity of a xx model can be checked using Euler's formula.
- Given a model, one can mathematically determine whether ... or ...
- Equations for xx need to be derived and implemented in the system.
- A number of heuristic rules have been developed for
- Optimum .. techniques can be made more reliable by ... so that
- An algorithm based on the characteristic ... is used to determine
- Euler's formula states the following:
- The completed model should agree with the formula.
- For manufacturing purposes, a detailed and precise model of the object is necessary
- Engineering design models are very well defined; therefore,
- To keep the domain narrow enough to be implementable, yet wide enough to be useful.

Point of View

- from an implementation standpoint,
- From the point of view of this application,
- From this point of view, Zadeh suggested an inference rule named xxx (CRI for short).
- Information is the meaningful interpretation and correlation of some aggregation of data in order to allow one to make decisions.
- From a practical point of view, the computational aspects of an FLC require a simplification of the fuzzy control algorithm.
- The use of a hammer to insert screws, although partly effective, tends to distort, destroy, and generally defeat the purpose of using a screw [Kusiak AI Implications for CIM p.129]

Statistical analysis

- Data were analyzed by one-way analysis of variance comparing the three conditions (sham operation, ischemic AKI, and bilateral nephrectomy) at each time point. If significant F-statistic from analysis of variance existed, this test was followed by Dunnett post hoc multiple comparison procedure with sham operation as the control group. For all other comparisons, Student's t-test was used. A P-value of <0.05 was considered as statistically significant.
- Values are expressed as means±s.e.m. and significance was evaluated by Mann–Whitney U test using GraphPad Prism, version 4.0 software (GraphPad Software Inc., San Diego, CA, USA).
- All values are expressed as means±s.d. Statistical significance (defined as $P<0.05$) was evaluated using analysis of variance and Bonferroni t-tests, and the two-tailed Pearson's test, where appropriate.
- Data are expressed as mean±s.d., median and interquartile range, or frequencies, as appropriate. Variables who deviated from the normal distribution (positively skewed) were log-transformed (log10) before the correlation study.
- Data are represented as means±s.e.m. Student's *t*-test and multiple comparisons with *t*-test *post hoc* analysis of variance were used as indicated below, for the comparison of

morphological, immunohistological and functional parameters. Statistical significance was set at $P<0.05$.

- The primary efficacy parameter was defined as change in urinary protein excretion from baseline (week 0) to 12 months after treatment. The 12-month changes were tested against zero using the paired t-test. Secondary end points included 6-month changes in protein; the number with PR or CR at 6 or 12 months; and changes in glomerular filtration rate (GFR), serum albumin, and lipid profiles. Study sample size was based on the desire to have 80% power to detect a drop in urinary protein of at least 2.0 g day-1. Assuming a two-sided hypothesis test performed at a significance level of 0.05 and an s.d. of urinary protein change of 2.5 g, it was determined that 15 patients were required.2 Definition of remission status is according to the criteria established by Cattran et al.30 CR was defined as proteinuria <0.3 g per 24 h, PR as proteinuria ≤3 g per 24 h, and a >50% reduction in peak proteinuria and non-response as <50% reduction in peak proteinuria. Any patient reaching a CR or PR was considered a treatment success
- The statistical significance of differences for the mean values of cytokine concentration and T cell proliferation was determined with Student's *t*-test. Differences with a *P* value of less than 0.05 were considered significant.
- Statistical significance was determined by Student's *t*-test. Data with a *P* value of less than 0.05 were considered significant.
- *In vitro* and *in vivo* experiments were analyzed by the two-tailed Student's *t*-test, with *P* values less than 0.05 considered statistically significant.
- The significance of differences among groups was determined by Student's *t*-test and one-way analysis of variance (SigmaStat software; Jandel).

Comparisons

- As well, the pros and cons of these representations from a process planning point of view will be discussed.
- The method of using xx to implement xx described by Zadeh (1973) appeared more suitable
- As discussed [in the previous section]/[preciously],

Relation

- We can not invert F' directly because it defines a many-to-one mapping.
- The relationships appear very complicate
- Lifting tasks involve complex and imprecise relationship between the task variables and the human operator's characteristics.
- These methods are based on the relationship between ... and ...
- The fundamental concept of a fuzzy rating language is that we can establish a relationship among terms such as high, medium, and low, and then modify these relationships.
- This article will thus mention the latter as well as the former.
- The former two bear a close relation to a fuzzy Cartesian product.

Importance

- The emphasis is on an implementation of a general approach to rule based decision making.

Consideration / Attention

- Careful evaluation is necessary to ensure
- Such a formulation does not change further considerations.
- Considerable attention has been paid to
- Attention should be paid to an important finding of this investigation.
- Caution should be exercised in this process to avoid ...
- Primary consideration is given to ... components, though others can be accommodated
- After ... has been defined by ..., a carefully analysis is carried out/performed to determine
- A number of factors such as ...need to be taken into consideration before making the appropriate decision.
- It should be noted that
- It is important to point out that ...
- These considerations have heightened interest in the possibility of providing ...
- We should stress the fundamental importance of the xx

Results.

Advantages / Disadvantage

- One of the major advantages of this new measure of xx is that it can be applied to the experimental study of
- One advantage of using a .. is the ease of preparing it.
- It has a very fast decision making process
- All the algorithms involve mostly logical operations.
- It can be easily and without additional cost implemented in a microprocessor;based environment.
- It can reduce the waste of designing from scratch.
- The advantages of using a xx to represent xx are the following:
- However, xx is not without its shortcomings.
- In most cases, the xxx shows an improvement over the existing xxx.
- Compared to the existing xx, the impacts of the xx are generally reduced by 5% to 9%.
- The "best case" results shows a savings of 6% to 9%.
- Most of the existing works based on xx approach can only recognize a xx .
- Most of the above methods are computational expansive and limited to xx.
- Some other advantages of xx are the following:
- The problem is the limitation of this method to a limited domain of parts.
- It proved limited in application because it demanded precision in system modeling that was impossible in practice.
- There are advantages to be gained in the structuring of costs and benefits, the use of xx,
- The disadvantages of this method are also disadvantages of conventional xx approaches.
- This combines the best features of both techniques

- Hopefully, this tool can be as the reference framework of for developing a xx platform, and helping the administration, marketing, and knowledge management activities in virtual communities.

Results

- An improvement on the result shown above can be made by based on the data provided
- Recent work has identified
- Time-dependent changes of
- Cyclosporine-induced cell death is triggered by a non-classical phosphoinositide 3-kinase and does not require ERK activation.
- Much of the current work on
- Discussion of these theories is beyond the scope of this review
- Based on the information contained in this
- The result can be categorized into nine classes
- The results are illustrated by an example
- The experimental results for each xx time are reported in Table 2.
- From the results obtained so far, it seem that
- Because of the inaccuracy of the ..., a conclusion cannot be drawn as
- Although much effort has been made to., this reality is far from completion.
- The results indicate that the total benefits are higher than the total costs.
- Their results may then serve as guidelines for lower level models, less fuzzy and more detailed.

Conclusion

- From the discussion, one may conclude that ...
- The first indication that
- In summary, we have shown that
- These results suggested that
- Our findings have shown that
- Our observations have provided
- This study reveals following five main findings:
- To our knowledge, this is the first immunohistochemical report of both PIM and HIFs in the diabetic kidney.
- As mentioned above,
- Moreover, we demonstrate that,
- A number of studies have elaborated a body of knowledge about
- There are findings to indicate that
- On a descriptive level, there is agreement that for
- One issue that became particularly evident from our study is
- Our data are in agreement with the findings and models presented by previous publications, in particular those of Jones *et al*
- According to our observations,
- …was not evident from our data

- It could therefore be concluded that
- our data illustrate that
- All these different aspects, as listed above, lead to
- In fact, it has already been speculated that
- It should be furthermore mentioned in this context that
- To identify and verify the essential factors triggering developmental renin expression, it is of interest now to study the development of intrarenal renin expression in mice with defined gene mutations, using the results of this study as a reference system.
- In conclusion, using several approaches, we demonstrated that
- But we have not provided formal proof yet that
- In summary, our results suggest that
- In conclusion, we identify
- A better understanding of such regulatory systems may yield novel therapeutic approaches to glomerular diseases.
- Preliminary results of short-term studies (2 weeks) indicate that
- However, we believe that
- However, despite our extensive measurements of these parameters, we found no relationship between response and initial proteinuria, time to B-cell recovery, the development of antibodies to rituximab, B-cell count in the kidney tissue, nor the degree of tubular interstitial damage present. A clear conclusion for efficacy, however, cannot be obtained from an uncontrolled study, and definitive claims of efficacy should be reserved for rigorous, prospective, controlled, and randomized trials with the use of rituximab and that will also look for factors that may determine its efficacy in IMN.
- It has become increasingly clear that
- Form the above discussion, the conclusion can be reached that
- The conclusions drawn are also valid
- In conclusion to this, it becomes obvious that the problem of xx lies not only in...
- We have attempted to introduce some concepts associated with a theory of
- Considerable more work, hopefully, will be done in this area
- Employing the compositional rule of inference, the assessment of the xx compatibility in achieving prescribed xx projectiles in any level of the hierarchy is made possible.
- This paper has presented a theoretical and experimental study of the xx process and xx concept.
- The experimental research results will hopefully serve as useful feedback information for improvements for xx work.
- The scope of this contribution was to introduce a xx method.
- This has a tremendous impact on our understanding of
- Significant progress has been made in advancing RNAi therapeutics in a remarkably short period of time
- To date, little is known about…
- Irrespective of the mechanisms and the molecules –which are still largely unknown – involved in MSC functions, current data suggest that
- To better understand

Future Research (常用于 conclustion 的结尾)

- Thus, first extension of the approach could be,
- Further studies are needed to determine the importance of
- Some improvements to the scheduling aspect of the model may be brought through additional levels in the hierarchy for more detailed representation of the scheduling activity.
- It also unveiled new aspects of the role played by thyroid hormone in response to injury and tissue repair.
- In the near future, the ongoing clinical trials with siRNAs for macular degeneration and RSV may reveal the exciting potential of RNAi therapeutics as the next major class of drug molecules.
- In the next few years it should become clear whether
- It should be an exciting time for researchers seeking to harness this powerful endogenous pathway to treat human disease.

Acknowledgement

- The authors are grateful to the insightful work provided by Mr. John W. Harney and all of the fellows and students that contributed with some of the studies discussed in this article
- I would like to express my gratitude to all those who gave me the possibility to complete this thesis.
- I want to thank the Department of Transport of the former Interim Government of Namibia for giving me permission to commence this thesis in the first instance, to do the necessary research work and to use departmental data
- This work was supported by NIH grants AI058695 and AI056900. We thank members of the laboratory and our collaborators for many useful suggestions.
- We thank J. Maraganore and B. Greene for critical reading of the manuscript, M. Duckman for graphics assistance and A. Capobianco for word processing assistance.

Author contributions

- All authors were involved in experimental planning and data analysis and contributed to manuscript preparation.

Tables and Figures

- Figure 7-1 sketches these relationships.
- The graphical representation of these functions is shown in Figure 1.
- The xx may be depicted as in Figure 1.
- Figure x shows the schematic diagram of the
- Figure 1 though 2 provide a ... that
- the architecture of this expert system for .... is illustrated in Figure 2.
- Figure 2 gives the outline of an ... system
- Table shows the

- Time-dependent changes of
- as shown in Table 1 and 2
- This concept is illustrated in Figure 2
- At the top of Table xx are shown two blocks of data.
- Each table or matrix has constructs xx through xx as row;headings,
- xx through xx as column;headings.
- Time course of calculated renin immunoreactive tissue volumes during development of the mouse kidney. Data are single values per time point. They were derived from the kidneys shown in Figure 1.
- Yellow color indicates
- PCT3 cells were pretreated with 50 μM PD98059 or 10 μM U0126 for 30 min and then cells were stimulated with 25 μg ml^{-1} CsA for 6 h. ERK and PKB activation were analyzed by western blot using phospho-specific antibodies.
- ERK and PKB activation were measured by western blot using phospho-specific antibodies
- The data are the means of three different experiments taking the number of untreated cells (vehicle alone) as 100%.
- ERK and PKB activation were then analyzed by western blot with the corresponding phospho-specific antibodies. To ensure equal amount of protein in each lane, western blots against total protein were performed for ERK and PKB.
- The means of optical density (OD) values of the bands detected in western blots of three different experiments are plotted, taking the maximal value of phosphorylation of ERK and PKB as 100%.
- CT3 cells were treated with 25 μg ml^{-1} CsA or vehicle alone for 24 h and then fixed with paraformaldehyde and stained with Hoescht as indicated in Materials and Methods. Then cells were visualized by phase-contrast microscopy (upper panel) and fluorescence microscopy (lower panel).
- The data are the means of three experiments performed on different days taking the number of cells at the onset of the experiment as 100%.
- Rats were killed at 4 h and at days 1, 2, 4, 7, 9, 14, 21, and 28 after disease induction (n=9 each).
- $^{*}P$<0.05 versus non-nephritic rats.
- The expression of the CCN3 protein was detected by western blot analysis (n=4 each, PC, positive control, M, molecular weight markers).
- Original magnifications: ×200 in A, E, G, H; ×600 in B, C, D, F.
- Data are means±s.d. of four independent experiments.
- $^{*}P$<0.05 versus 0.5 h.
- PDGF-BB and -DD, but not PDGF-AA and -CC, induce a downregulation of *CCN3* mRNA as determined by real-time RT-PCR and normalized to *glyceraldehyde-3-phosphate dehydrogenase* mRNA.
- Cell number was counted in duplicate using a Malassez hemocytometer
- Data are means±s.d. of four independent experiments. $^{*}P$<0.05 versus cells treated with MCDB media for 24 h, #P<0.05.

- The curves are adjusted for history of coronary artery disease, CRP, and progression to ESRD.
- Both models are independent of age, gender, baseline GFR and proteinuria, and other clinical characteristics and traditional and nontraditional cardiovascular risk factors
- Correlation between serum bone-specific alkaline phosphatase (bAP) and serum PTH (n=99, P<0.0001, r^2=0.190)
- Concentration of serum FGF23 concentration before starting and at the end of the 4-h hemodialysis procedure in 23 patients, expressed as $\log_{10}$ values (n=23, P<0.0001, Student's paired t-test).
- Immunohistochemistry for the hypoxia marker pimonidazole (PIM) and HIF-2α; arrow=endothelial cell and CD=collecting duct
- Magnification: ×1000.
- A table of .. is developed and significant recommendations are made.

CONJUGATION

To Indicate Addition

- additionally, again, also, and then, as can be easily understood, besides, equally important, especially, finally, for the same reason, first, further, furthermore, in addition, last, likewise, moreover, next, second, third, too, evidently, obviously, roughly speaking, broadly speaking

To Indicate Cause and Effect

- accordingly, as a result, consequently, for this reason, hence, in short, otherwise, then, therefore, thus, truly

To indicate Comparison

- in a like manner, likewise, similarly , alternatively

To Indicate Concession

- after all, although this may be true, at the same time, even though, even so , I admit, naturally, of course

To Indicate Contrast

- and yet, at the same time, but, for all that, however, yet, in fact, in contrast, in the real life, in spite of, nevertheless, notwithstanding, normally, on the contrary, on the other hand, still, traditionally, rather, unfortunately,

To Indicate Time Relationships

- after a short time, afterwards, as indicated earlier, as long as, as soon as, at last, at length, at the moment, at that time, at the same time, before, earlier, currently, immediately, in the meantime, in recent years, lately, later, meanwhile, often, of late, presently, recently, soon, shortly, since, thereupon, temporarily, therefore, until, when, while

To Indicate Special Features or Examples

- for example, for instance, incidentally, indeed, in fact, in other words, in particular, in practice, specifically, that is, to illustrate, in this respect, theoretically, as mentioned before / above

To Indicate Summary

- in brief, in conclusion, in short, in summary, on the whole, to conclude
- , in general, to summarize, to sum up, as a result, ultimately,

VERB PHASE

- build a .. model
- have been described to
- To assess the functional role of
- we detected
- build up the key link
- Although a number of studies have identified predictors of
- began a new era in ...
- can be regarded as / achieved / used to/for / found / obtained through
- can result in
- carries out ... tasks
- production information in order to simultaneously
- contains all information necessary to describe
- do not make use of production information
- deals with
- end with failure
- fetch the information from the model directly
- has great potential / yet to be resolved / spurred the development of /
- been recognized as
- site component / cable of / currently implemented for / demonstrated by an example / finally reached / made equal to / equivalent to / more suitable to / oriented to / interpreted as / pointed out / potentially of great benefit in the complex task of / shown in / used to effectively guide the search
- makes use of
- was significantly higher as compared to
- To assess disease-specific alterations, we measured
- To specifically assess the relationship between
- make up
- To determine if
- This statistically significant difference still
- They positively correlated with
- Present
- Our results are consistent with our recent data that suggest that

- contribute to
- meets the needs of real life production,/ the current demands placed upon it
- must be justified
- point out
- play an important role
- relates to
- rely on
- We performed
- satisfy the needs
- determine the total requirements for the ...
- uses ... as a key to search for...
- without relying on
- will be available/ performed/ overlooked

NOUN PHASE

- a basic technical function of
- a significant reduction of
- drug variability
- a critical need
- negatively correlated with
- a key / principle feature of
- a substantial impact on
- an intensive review was conducted
- The analysis of the correlations by
- an increasing need for expanding the application of ....
- an important component / function / aspect / issue
- each rule is numbered in sequence
- each of these involves
- for this calculation, it is necessary to define
- in the physical environment / integration of
- in the reality of situations where …
- many aspects of
- most past efforts have been spent on ...
- common sense to a well studied and documented technical field.
- sources of additional information on ... are listed
- systematic and rationally structured format
- the basis on which a range of ...operations can be established is shown
- THE basic philosophy / principles of / key element / general hypothesis / candidate list of / concept of ... has attracted wide interest / function is concerned with / heart / impact / nature / role / task of / kernel functions
- the number of parts needed to
- the above statement means that
- the output data is passed to

- the proposed method / underlying principle
- the recommendations made in this report, if implemented, should
- this information resides in
- this process is composed of ... different ... operation
- along with the use of
- concerning general aspects of
- due to
- for later use in generating...,
- in turn,
- it can be claimed/concluded that
- it demonstrates the decisions required of
- it also provides information to ..
- it becomes essential to
- let ... be the probability that
- once... is written, it is compiled into...
- suppose it is observed that
- this is because
- this results in a
- upon completion of the ... analysis,
- when the knowledge is of mathematics or quantum physics, it will also be
- recorded in books and papers
- selection of rules for using the tools, for generating operation plans,
- is another matter of preference, since practice varies greatly.
- for the sake of convenience
- correct decision to be reach
- keeping the number of rules to a minimum.
- a good process plan will result exhibiting several characteristics:
- practical solutions
- because of rather small job lot sizes
- Backward reasoning can be used to answer the question "should milling tool be select" different level of knowledge in the realm of process planning