RENAL & TRANSPLANTATION IMMUNOLOGY & PATHOLOGY

4 NOV 1996

Volume 1

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علم مناعة الكلية وزراعة الأعضاء

AHB
4 NOV 1996

الدكتور أحمد مختار
دكتوراه طب وعلاج

تقديم

يسعدنى ان يكون هذا المجلد حصيلة تشجيع ورعاية سيدى حضرة صاحب السمو الشيخ عيسى بن سلمان ال خليفة امير البلاد المفدى حفظه ا لله ورعاه، حيث وضع سموه النبراس لهذا المشروع والذى يستغرق سنوات عديدة، راجيا ان يكون اضافة جديدة للمكتبة الطبية ولنهضة وطننا الغالى.

يحتوى هذا المجلد الاول للتطورات الحديثة للكلية وزراعة الاعضاء على مجموعة من الدراسات والبحوث الطبية المعملية والاكلينيكية التى اجريت منذ عام ٨٨ – ٩٤ م فى البحرين والمملكة المتحدة، ولقد قدمت فى المؤتمرات الطبية والنشر فى المجلات الطبية المتخصصة.

PREFACE

The scientific achievement can not be preserved if it is not recorded and published. This first volume of advamcement in Renal and Transplantation includes some studies which were done from 1988 - 1994 of my pre-doctoral, PhD and post-doctoral period, enhanced with my 12 years studies in the field of medicine,. Four of these studies were submitted in the international conferences, two were published and others are sent for publication. Four of these studies were done by collaboration with consultants from Ministry of Health and BDF hospital.

This work could not been ahieved without the encouragement and care I recieved from his HIGHNESS SHAIKH ISA BIN SALMAN AL KHALIFA, this care which his highness promised to continue and despite all difficulties I am proud that it is finished on time.

This volume is divided into four parts: Pharmacology, Pathology, Biochemistry and Transplantation Immunology.

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February, 1995

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PHARMACOLOGY

EFFECT OF CYCLOHEXAMIDE, TUNICAMYCIN AND ACTINOMYCIN D ON MP 84 EXPRESSION BY MESANGIAL CELLS

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تاثير سيكلوهكساميد ، تيونيكاميسين
واكتينوميسين د على انتاج مادة
ام بى ٨٤ بواسطة خلايا الميزانجيال

ام بى ٨٤ بروتين فريد تنتج استجابة لكل المواد المنشطة حيث تم اكتشافها وعزلها بواسطة الدكتور احمد مختار (جامعة ابردين عام ١٩٩٤) ، وهى تنتج بواسطة خلايا الميزانجيال المنشطة وانسجة مرضى الكلية فقط .

هذه هو اول دراسة لميكانيكية انتاج هذه المادة بواسطة اضافة العقاقير الاتية : سيكلوهكساميد ، تيونيكاميسين واكتينوميسين د ، ولقد بينت الدراسة بان كل من العقارين سيكلوهكساميد واكتينوميسين د تمنعان انتاج مادة ام بى ٨٤

ABSTRACT

MP 84 is a novel protein synthesized in response to all cytokines. this antigen is expressed only in stimulated mesangial cells and diseased kidney sections but not in the normal kidney sections (Mukhtar A, 1992 & 1994). This is the first study of the mechanism of MP 84 expression by stimulated mesangial cells by incubating cells with cyclohexmide (inhibitor of protein synthesis), tunicamycin (inhibitor of transcription) and actinomycin D (inhibitor of N-linked glycosylation). Anti phosphatase anti alkaline phosphatase (APAAP) was used. This study shows that both transcription and translation are required for expression since both cyclohexamide (inhibitor of protein translation) and actinomycin D (inhibitor of transcription) inhibited the target antigen.

INTRODUCTION

MP 84 is a novel protein synthesized in response to all cytokines. this antigen is expressed only in stimulated mesangial cells and diseased kidney sections but not in the normal kidney sections (Mukhtar A, 1992 & 1994). This is the first study of the mechanism of MP 84 expression by stimulated mesangial cells by incubating cells with cyclohexmide (inhibitor of protein synthesis), tunicamycin (inhibitor of transcription) and actinomycin D (inhibitor of N-linked glycosylation). Anti phosphatase anti alkaline phosphatase was used. This could help in the inhibition of MP 84 expression by mesangial cells.

MATERIALS AND METHODS

Mesangial cells are incubated with cyclohexmide (inhibitor of protein synthesis), tunicamycin (inhibitor of transcription) and actinomycin D (inhibitor of N-linked glycosylation) for 30 minutes prior to addition of 10U/ml IL-1. The cells were co-incubated with IL-1 and the separate inhibitors for 60 minutes and then stained by APAAP.

ALKALINE PHOSPHATASE ANTI-ALKALINE PHOSPHATASE IMMUNOSTAINING (APAAP) USING MULTIPLE CHAMBER SLIDES

Mesangial cells were passaged to Lux multiple chamber slides and grown until they were confluent. The culture medium was poured off and cells washed with PBS. To fix the cells 0.2ml 0.025% glutaraldehyde was added to each well for 20 minutes at room temperature, and the wells were then washed 3 times with TBS. Monoclonal antibody MP 84 diluted in TBS was addded to each well (OX-8, TBS, and RF10 were used as negative controls), and incubated for 1 hour at room temperature. The slides were then washed 3 times with TBS. Rabbit anti-mouse Ig (100μl) was added at room temperature, prior to three washes with TBS. After dilution 1/40 in TBS 100μl of mouse APAAP complexes (Dako) was then added and incubated for 30 minutes at room temperature. The slides again washed 3 times with TBS prior to the addition of 200μl of freshly prepared substrate, veronal acetate buffer with naphthol AS-MX phosphate, levamisole and fast red salt, to each well and incubation for 15-20 minutes at room temerature. The slides were washed with distilled water 2-3 times to stop the reaction then

counterstained with haematoxylin, and rinsed with Sco'ts tap water to intensify the blue colour of the nuclei. Slides were viewed under the light microscope using aqueous mountant.

RESULTS

This is fisr study of the mechanism of MP 84 expression by mesangial cells by incubating cells with cyclohexmide (inhibitor of protein synthesis), tunicamycin (inhibitor of transcription) and actinomycin D (inhibitor of N-linked glycosylation) for 30 minutes prior to addition of 10U/ml IL-1. The cells were co-incubated with IL-1 and the separate inhibitors for 60 minutes and then stained by APAAP. Antibody 84 did not stain cells incubated with cycloheamide and actinomycin but stained cells incubated with tunicamycin. Mesangial cells were 90% viable following 90 minutes incubations with these inhibitors in control experiments, assessd by ethidium bromide dye exclusion and fluorescence within the nuclei of cells after incubation with acridine orange.

DISCUSSION

In this study of a novel antigen (MP 84) expressed by cultured glomerular mesangial cells in response to stimulation with IL-1, the mechanism of its expression was studied. The protein is virtually specific to stimulated mesangial cells and diseased kidney sections (Mukhtar A, 1992 & 1994). The protein is synthesized de novo within 30 minutes of administration of IL-1 to cultured cells, wether cycling orarrested.

This study shows that both transcription and translation are required for expression since both cyclohexamide (inhibitor of protein translation) and actinomycin D (inhibitor of transcription) inhibited the target antigen, also shows that MP 84 expression could be inhibited by admininstration of actinomycin D and cyclohexamide to cultured cells.

REFERENCES

-Mukhtar A. Study of glomerulonephritis using monoclonal antibodies against mesangial matrix. PhD thesis. University of Aberdeen: 1992. UK.

-Mukhtar A. Expression of MP84 in the biopsies section of benign and malignant tumours. Accepted for publication in Hamdard Medicus: 1994.

-Power D A, Mukhtar A and Jones M C. Production of monoclonal antibody to stimulated mesangial matrix. Kidney International. Abstract. 1992; 42, (3): 703-704.

PATHOLOGY

MP84 EXPRESSION IN PYELONEPHRITIS AND HYDRONPHROSIS KIDNEY SECTIONS

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وجود مادة ام بى ٨٤ فى انسجة المرضى
المصابين بالتهاب حوض الكلية

مادة ام بى ٨٤ بروتين فريد تفرز فى انسجة المرضى المصابين بالتهابات الكلية البدائية وخلايا الميزانجيال المنشطة ولا توجد فى الانسجة الاخرى.

هذه اول دراسة لوجود مادة ام بى ٨٤ فى انسجة المرضى المصابين بالتهاب حوض الكلية، ولقد ثبت وجود هذه المادة ولو ان توزيعها تختلف عن التهابات الكلية الاخرى، وهذه لها اهمية فى نوعية المرض والاعراض.

Abstract

MP84 is a novel antigen expressed in the primary glomerulonephritis kidney biopsy section and stimulated mesangial cells and matrix but not in the normal kidney and other tissues sections or any other cell lines tested except BeWo choriocarcinoma cell line. This is the first report of MP84 expression in pyelonephritis and hydronephrosis kidney section. The distribution of the antigen is different from the primary glomerulonephritis kidney sections studied previously sush as membranous, minimal change, focal and segmental, and IgA nophropathy. This may have an important correlation with the pathological concept underlying the disease.

Introduction

MP84 is an antigen detected by a very specific monoclonal antibody 84 which is specific for primary glomerulonephritis such as IgA nephropathy, focal and segmental, membranous and minimal change (Power & Mukhtar 1991, and Mukhtar 1992). It is also found in stimulated mesangial cells with cytokines such as interleukin-1 (IL-1), interleukin-6 and tumour necrosis factor (TGF) and also found in BeWo choriocarcinoma cell line, and it was not found in any other cell line studied. MP84 has a molecular weight of 97, 125 and 160 KD which is a heavy three bands protein chains produced within 15 minutes by mesangial cells stimulated with certain cytokines in vitro and secreted into the matrix within 30 minutes. This the first attempt to study the expression of MP84 in pyelnephritis and hydronephrosis diseased kidney sections. Pyelonephritis is defined as the disease process resulting from non specific bacterial infection of the kidney and upper urinary tract. It is recognized in acute and chronic forms, both of which are commonly associated with urinary tract obstruction.

Materials and Methods

Immunostaining of Tissue sections Fixed with Paraformaldehyde: Frozen sections were fixed for 20 minutes 2% with (wt/vol) paraformaldehyde in PBS, incubated for 20 minutes, quenched with amonium chloride, and permeabilized for 1 hour with PBS containing 0.01% (wt/vol) saponin and 0.25% (wt/vol) bovine serum gelatinin (PBS/sap/gel. Wells were incubated with monoclonal antibodies supernatants, with ascitic fluid or serum diluted in PBS/sap/gel for 1 hour (OX-8, TBS and RF10 were used as negative controls), washed 3x with PBS/sap/gel and 100 1 of anti mouse Ig FITC conjugate (TACO, Inc), which has been diluted in 1/80 in Evans blue dye in PBS/sap/gel was applied. After incubation for 30 minutes at room temperature the slides were washed 3x with PBS/sap/gal and sealed under 10% glycerol in PBS containing 1,4 diazabicyclo-(2,2,2) octane (Polyscience, Inc) to minimize quenching, and examined under the fluorescent microscope -Olympus, BH2- (Burkadt,1989 and Mukhtar, 1992)).

Results

Immunohistopathlogy

Showed The expression of MP84 antigen as following: Proximal tubules showed no expression of the antigen while some of distal tubules were of high (+3) antigenic materials localization within the tubules and granular deposition of glomerular epithelial cells materials. Vesseles: Nil, interstitial cells: Nil, Glomerular basement membrane (GBM): lamina rara interna : +1, lamina rara externa: +2 which appears widened and loosely with fine granules, Mesangial cells: +1, mesangial matrix: +2 with some expansion. Glomerular epithelial cells : +1 and Glomerular endothelial cells: +1. The glomeruli are within normal limits and no indication of any antigenic allocation within the pelvis.

Discussion

The immunofluorescense examination of the kidney section of this patient with pyelonephritis and hydronephrosis showed the expression of MP84 mainly in the glomerulus and some tubules. This pattern of the antigenic expression is different from the previous studies for primary glomerulonephritis diseased kidney sections such as IgA nephropathy, minimal change, focal and segmental and membranous (Mukhtar, 1992). The normal appearance of the glomeruli is in the parallel with acute pyelonephritis whereas the glomeruli are remarkedly well preserved and unaffected by the inflammatory process (Tisher and Benner, 1989).

It is known that the glomeruli in pyelonephritis have different stages of pathology changes from normal to complete hyalinization Glynn et al (1971) reported that strains of E. coli rich in K antigen are especially associated with kidney infection. Ascending infection from the bladder is by far the most common route for the infection of the kidney. Intrarenal reflux acts as a mechanisms whereby pathogenic organisms are introduced directly into selected areas of paranchymes, the extent of which depends on papillary morphology and added factors such as abnormal bladder and obstruction (Tisher and Benner, 1989).

The non-specific bacterial infection and cellular injuries could lead to production and secretion of growth factors such as IL-1, IL-6 and TGF and these initiates the autocrine and paracrine production of the cytokines which might stimulates the mesangial cells in the kidney to pruduce MP84 antigen (Power, Mukhtar and Jones, 1992). The pattern of the distribution of this antigen in

different kidney diseases is not understood. It was reported that it is closely associated with the histopathological concept of the primary glomerulonephritis (Mukhtar, 1992). The distribution of MP84 in the glomerulus and the distal tubules could be correlated with the filtration process or the non-specific bacterial infection, but the abscence of the antigen in the interstitial cells indicates that these cells are not involved in the production and secretion of MP84 antigen. Weather this antigen is produced and secreted by the mesangial cells in the kidney is not Known, but it is known that it is not produced by any other cell line in vitro and it was not found in normal and other tissues kidney sections studied.

This study shows that MP84 is pruduced and secreted in the pyelonephritic and hydronphrosis kidney section in addition to the primary glomerulonephritis kidney sections. More histopathological studies needed for the expression of this antigen in kidney sections of systemic diseases.

References

Burkhadt J K, Hester S and Argon Y. The glycoprotein of VSV accumulates in a distal golgi compartment in the presence of CCCP. J Cell Sci. 1989; 92: 643-645.

Glynn AA, Brumfitt W, and Howard CJ. Antigens of Escherichia coli and renal involvement in urinary tract infections. Lancet. 1: 514-516, 1971.

Mukhtar A. Study of glomerulonephritis using monoclonal antibodies to mesangial matrix. PhD thesis. University of Aberdeen. 1992.

Power D A, Mukhtar A, and Jones M C. Production of monoclonal antibodies to stimulated mesangial cell matrix (Abstract). Kidney International. 1992. 41 (3): 703.

Tisher C C and Benner B M. Renal pathology with clinical functional correlation. Vol I. J. B. Lippincott Company. 1989. p 777-808.

MP84 ANTIGEN EXPRESSION IN BIOPSY SECTIONS OF PATIENTS WITH BENIGN AND MALIGNANT TUMOURS

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وجود مادة ام بى ٨٤ فى انسجة
المرضى المصابين بالاورام الحميدة والخبيثة

هذه الدراسة تبين بان مادة ام بى ٨٤ تنتج فى انسجة المرضى المصابين بسرطان خلايا الكلية ولا تنتج فى اى من الانسجة الاخرى للاوراو الحميدة او الخبيثة الاخرى ، وكما هو معروف فان هذه المادة تنتج فى انسجة المرضى المصابين بالالتهابات المختلفة ، وكذلك فى الخلايا بى و السرطانية فى المزارع النسيجية، واحداث المرض قد يكون بسبب افراز المواد المنشطة من قبل خلايا الكلية .

INTRODUCTION

Tumours from defined and those from which etiological hypothesis can be deducted such as environmental origin play as an important factors in the growth and development of tumours. The problem of generation of neoplasms from initiated cells is a central one in carcinogensis. Wether a cell is initiated by germinal mutation or by somatic mutation, that event is insufficient to prodice a malignant tumour (Knudson A G, 1981).

Growth factors and growth factors receptors are may be involved in the abnormal growth charateristic of cancer, and both entities are encoded by known proto-oncogens.

MP 84/15 is a protein synthesyzed in response to several cytokines (EGF, PDGF, IGF-1, IL-1, IL-6, FGF and TGF). It is produced by the mesangial cells within 15 minutes and secreted into the matrix within 30 minutes when the cells are stimulated in vitro. This antigen is present only in diseased kidney sections and choriocarcinoma BeWo cell line (Mukhtar A, 1991 & 1993). Its expression in tumour tissues sections is unknown.

MATERIALS AND METHODS:

Tissue sections

Tissues sections of the following tumours were used in the study, and FITC was performd: Metastatic carcinoma, Retinblastoma, Basal cell carcinoma, Benign prostatic hyperplasia, Malignant lymphoma (Intermediate grade), Conjunctival squamous cell carcinoma, Transitional cell carcinoma, Dermatofibroma, Acute lymphoblastic leukaemia, Basal cell carcinoma (Skin), Squanous carcinoma (Cervix), Transitional cell carcinoma (Urinary bladder), Serous cystadenoma (Ovary), Subependymoma (Cerebellum), Pyogenic granuloma, Non-Hodking's lymphoma (Rectum), Squamous cell carcinoma (Uterine cervix), Carcinoma (Pleura), Mucinous carcinoma (Stomach),Malignant vasoformative tumour, Fibroadenoma (Breast), Papillary cell carcinoma Grade II (Bladder), Invasive cell carcinoma (Cervix), Squamous cell carcinoma (Gum),Renal cell carcinoma, adenocarcinoma (Skin), Squamous cell carcinoma grade II (Tongue), Benign juvenile melanoma "Spitz Naevus" (Skin), Renal cell adenoma.

Immunostaining of Tissue sections Fixed with Paraformaldehyde: Frozen sections were fixed for 20 minutes 2% with (wt/vol) paraformaldehyde in PBS, incubated for 20 minutes, quenched with amonium chloride, and permeabilized for 1 hour with PBS containing 0.01% (wtlvol) saponin and 0.25% (wt/vol) bovine serum gelatinin (PBS/sap/gel. Wells were incubated with monoclonal antibodies

RESULTS:

There was no staining of any tissue section used in this study except renal cell carcinoma as follows: All tissues of the renal cell carcinoma were not stained except the glomerulus. Glomerular basement membrane (+3), mesangial cells (+2), mesangial matrix (+3), epithelial cells (+2) and endothelial cells (+2). Generally adense granular stainning of the glomerular basement membrane and cells was noted.

DISCUSSION:

This study showed that MP84 is expressed in the kidney biopsy sections with renal cell carcinoma. The expression of the antigen was strictly located in the glomerulus, with strong glomerular basement membrane and mesangial cell and matrix expression. This pattern of staining is different from MP84 expression in primary glomerulonephritis kidney biopsy sections such as IgA nephropathy and membranous. It was noted that this antigen is not expressed in other malignant tumours sections studied.

Cytokines are produced in the response to the mesangial cellular injury (Sedor JR, 1992), and these alters the mesangial and extracellular matrix components (Sterzel RB, Schulze-Lohoff E and Marx, 1993). The molecular and biochemical events, which mediate cytokine-induced transition are poorly understood. This process involves induction of aspecific gene set by a network of a short-term signals generated by interaction of the cytokine with the binding domain of its receptor.

The expression of MP84 antigen in the renal cell carcinoma kidney section could be in by same process as in primary glomerulonephritis kidney (Mukhtar A, 1993). Its relation to its expression in BeWo choriocarcinoma cell line is not understood since no section of choriocarcinoma was used in this study. However this study shows that MP84 antigen is only expressed in the diseased kidney section including malignant tumours of the renal such as renal cell carcinoma.

References

Burkhadt J K, Hester S and Argon Y. The glycoprotein of VSV accumulates in a distal golgi compartment in the presence of CCCP. J Cell Sci. 1989; 92: 643-645.

Glynn AA, Brumfitt W, and Howard CJ. Antigens of Escherichia coli and renal involvement in urinary tract infections. Lancet. 1: 514-516, 1971.

Mukhtar A. Study of glomerulonephritis using monoclonal antibodies to mesangial matrix. PhD thesis. University of Aberdeen. 1992.

Power D A, Mukhtar A, and Jones M C. Production of monoclonal antibodies to stimulated mesangial cell matrix (Abstract). Kidney International. 1992. 41 (3): 703.

Sendor JR. Cytokines and growth factors in renal injury. Semin Nephrol. 12: 428-442, 1992.

Sterzel RB, Schulze-Lhoff E and Marx M. Cytokines and mesangial cells. Kidney Int; 39: 26-31, 1993.

Tisher C C and Benner B M. Renal pathology with clinical functional correlation. Vol I. J. B. Lippincott Company. 1989. p 777-808.

MESUREMENT OF CULTURED MESANGIAL MATRIX PROTEIN PRODUCTION BY BRADFORD TECHNIQUE

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قياس انتاج بروتينات وسادة خلايا
الميزانجيال بواسطة طريقة برادفورد

تبين من الدراسات بان تقلص خلايا الميزانجيال قد تؤدى الى التكيف مع مراكزالترشيح فى الكلية استجابة للتغيرات فى السوائل الخارجية للخلايا على حسب تقلص الخلايا وذلك لتنظيم ديناميكية الترشيح وهى ضئيلة.

وسادة خلايا الميزانجيال تشبه فى الشكل الخارجى (المورفولوجى) غشاء المبطن لمراكز الترشيح ولكنها تختلف عنها كيميائيا ، هذه الدراسة تقيس معدل البروتينات المنتجة للخلايا الميزانجيال المنشطة والغير المنشطة بواسطة طريقة برادفورد .

لقد بينت هذه الدراسة بان انترليوكين-١ تزيد من معدل البروتينات بينما بى ام ا تقلل مقارنة مع الخلايا الغير المنشطة ، وهذه بدورها تغير من مساحة الفتحات وخصائص وسادة الخلايا والترشيح مما تؤدى الى تغير فى طبيعة امراض الكلية وتطورها .

ABSTRACT

It has been suggested that mesangial cell contraction can adapt the glomerular tuft in response to variations in extracellular volume although the relevance of mesangial cell contraction for the regulation of glomerular haemodynamics appeared to be small. Mesangial matrix is an intercellular substance morphologically similar to but biochemically distinct from glomerular basement membrane. This atudy measures the amount of stimulated and non-stimulated mesagial cell protein production in culture using Bradford technique.

This study shows the effect of cytokines on alteration of extracellular matrix proteins produced by mesangial cells, and it shows that IL-1 increases the amount of matrix priteins while PMA decreses the amount of matrix proteins compared to the non-stimulated cells. these in turn could lead to the alteration of pore size, matrix properties and filtration, and thus influence the development and progression of glomerular diseases.

INTRODUCTION

It has been suggested that mesangial cell contraction can adapt the glomerular tuft in response to variations in extracellular volume although the relevance of mesangial cell contraction for the regulation of glomerular haemodynamics appeared to be small. Furthermore, the reduction in filtration area, although prominent, cannot fully account for the considerable decreases in the ultrafiltration coefficient observed by others in acute and chronic studies (Elger et al, 1990). Mesangial matrix is an intercellular substance morphologically similar to but biochemically distinct from glomerular basement membrane. Muhktar A (1992) mentioned that cytokines alters extracellular matrix proteins of certain adherent cell lines with particular reference to mesangial cell. This atudy measures the amount of stimulated and non-stimulated mesagial cell protein production in culture using Bradford technique.

MATERIALS AND METHODS

ISOLATION OF MESANGIAL EXTRACELLULAR MATRIX

Matrix was harvested from confluent mesangial matrix after removal from media. Cell dissociation medium (2ml, Sigma) was added to the flask which was incubated at 37°C/5%CO_2 for 10-15 minutes. RF10 was then added to the total of 10ml, the flasks agitated and the contents transferred to a sterile centrifuge tube. This process left extracellular matrix in the flask, the cells removed in RF10 were centrifuged and used in further cultures. Ectracellular matix (ECM) layers were eashed with phosphate buffered saline pH 7.3 (PBS) three times and the flask examined by phase-contrast microscope to ensure removal of all cells: if any remained more cell dissociation medium was used. The matrix was solubilized by incubation in 2.5ml (for an 80 cm^2 flask) 0.1% SDS at 37°/5% CO_2 for 15-20 minutes then vigoriously agitated for at least one minute. The solution containing matrix could be used directly or stored. Prior to storage at -20° four parts acetone were added to one part SDS to pericipitate protein.

MATRIX PROTEIN MEASUREMENT (BRADFORD ASSAY)

To 0.1ml of matrix protein solution (0-40μg) was added 0.9ml of reagent 0.4% Coomassie blue G250 in 3,5% $HCLO_4$. The mixture was left at room temerature for 5 minutes before determining optical density (OD) in a spectrophotometer at 595nm or 620nm. The determined OD was plotted against concentrations of a standardized at least very two weeks against BSA.

PREPARATION OF THE REAGENT

10ml distilled water

0.35ml $HCLO_4$

0.004ml Coomassie blue

Stirred for 1 hour then filtered.

GROWTH FACTORS USED

rIL-1 (Boehringer) and PMA (Boehringer).

RESULTS

This study shows the effect of cytokines on alteration of extracellular matrix proteins produced by mesangial cells, and it shows that IL-1 increases the amount of matrix proteins while PMA decreses the amount of matrix proteins compared to the non-stimulated cells (Table 1.1).

The mean amount of matrix protein pruduced by one cell of non-stimulated mesangial cells was 0.73 ng/ml matrix protein/cell, it was more than mean amount of matrix protein/cell of mesangial cells stimulated by PMA, but less than the mean amount of matrix proteins/cell of mesangial cells stimulated by IL-1.

DISCUSSION

This study shows the effect of cytokines on alteration of extracellular matrix proteins produced by mesangial cells, and it shows that IL-1 increases the amount of matrix priteins while PMA decreses the amount of matrix proteins compared to the non-stimulated cells. It has been suggested that mesangial cell contraction can adapt the glomerular tuft in response to variations in extracellular volume although the relevance of mesangial cell contraction for the regulation of glomerular haemodynamics appeared to be small (Elger et al, 1990). Mukhtar A (1992) mentioned that cytokines alters the extracelluar matrix proteins produced by mesangial cells.

The study shwed that cytokines aletered the production and secretion of extracellular matrix proteins, these in turn could lead to the alteration of pore size, matrix properties and filtration, and thus influence the development and progression of glomerular diseases.

REFERENCES

-Mukhtar A. 1992. Study of glomerulonephritis using monoclonal antibodies against mesangial matrix. PhD thesis. University of Aberdeen. UK.

-Elger M, Sakai T and Kriz W. Role of mesangial cell contraction in adaption of glmerular tuft to changes in extracellular volume. European J Physiology. 1990; 415: 598-605.

Table 11 Measurement of cultured mesangial cells protein production by Bradford technique

I) Non-stimulated cells

Flask	M/P	NC	M/P/M
	mg/ml10.i%SDS	$x10^6$	ng
A	45	62	0.73
B	40	57	0.57
C	30	44	0.68
D	55	68	0.81
E	60	87	0.70

Mean=0.73 ng/ml matrix protein/cell.

II) Stimulated with 10^{-5}M PMA

A	30	47	0.68
B	25	52	0.48
C	35	68	0.51
D	20	31	0.66

Mean=0.57 ng/ml matrix protein/cell.

III) Stimulated with rIL-1ß (10 U/ml RF10)

A	50	54	0.93
B	60	83	0.72
C	40	56	0.71
D	65	88	0.74

mean 0.76 ng/ml matrix prtein/cell.

M/P= matrix protein, NC=number of cells from 80 ml flask, M/P/M= matrix protein per one mesangial cell. Cells grown in 80 ml flask for 48 hours with 30 ml RF10.

SDS GEL ANALYSIS OF MATRIX OF ADHERENT CELL LINES WITH PARTICULAR REFERENCE TO MESANGIAL CELLS

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التحليل الكيميائى لوسادة الخلايا
وبشكل خاص لخلايا الميزانجيال

خلايا الميزانجيال تنتج وتفرز كمية مختلفة من البروتينات وهذه تزيد بواسطة المواد المنشطة ، وتمت هذه الدراسة لمعرفة الوزن الجزيئى للبروتينات المنتجة للخلايا الميزانجيال ، هب جى ٢ ، بى و ، و بى اج ك المنشطة والغير المنشطة .

النتائج بينت بوجود رابطة جزيئية مشتركة بوزن جزيئى مقداره ٦٥ - ٧٠ ك د فى وسادة جميع الخلايا المنشطة والغير المنشطة ، بينما تم الحصول على رابطة من وزن جزيئى مقداره ١٥٠ ك د فى خلايا المزانجيال و بى و المنشطة فقط . هذه الدراسة توضح تاثير الواد المنشطة على انتاج البروتينات المختلفة .

ABSTRACT

Cultured mesangial cells produce a variety of extracellular matrix proteins and secretion are increased by cytokines. To identify the molecular weight of proteins in the matrix of stimulated and non-stimulated mesangial, BeWo, Hep G2 and BHK cells was studied using SDS and proline gel analysis. The results showed a common band of 65-70 kd in the matrix of both stimulated and non-stimulated mesangial cells and other cell lines, while a unique 150 kd band was obtained in the matrix of stimulated mesangial and BeWo only. These data indicate the effect that cytokines have on the expression of different proteins.

INTRODUCTION

Mesangial matrix is an intercellular substance morphologically similar to but biochemically distinct from glomerular basement membrane. The main extracellular matrix proteins are collagen, laminin, proteoglycan, vitronectin, actin and fibronectin. SDS gel analysis was used to identify the molecular weight of proteins in the matrix of stimulated and non-stimulated mesangial, BeWo, Hep G2 and BHK cell lines. It was shown that cytokines alters extracellular matrix proteins of mesangial, Hep G2, BeWo and BHK cell lines (Mukhtar A, 1992).

MATERIALS AND METHODS

Matrix of adherent cell lines was isolated

SDS-PAGE ELECTROPHORESIS

This was performed using Bio-Rad apparatus and method described by Laemmle (1979).

The seperating gel was poured, covered with 0.1% SDS buffer and incubated for more than 1 hour or overnight at room temperature. The stacking gel was poured on to the top of the seperating gel. Polymerisation occurred at room temperature over 45-60 minutes. The extracellular matrix was prepared and boiled in SDS sample buffer (reducing or non-reducing). Molecular weight markers (Amersham Rainbow standards or Bio-Rad molecular weight standards) were prepared in similar buffers. The samples and standards were loaded onto the wells of the gel. 200μl bromphenol blue was added to the upper chamber which was filled with running buffer. After the run was complete, the gel was removed and treated in one of the following ways:

a) Bloted to nitrocellulose membrane (Western blot).

b) Silver stained (Bio-Rad silver staining kit).

c) Coomassie blue stained.

d) Dried on gel drier and autoradiographed.

PROLINE ANALYSIS OF EXTRACELLULAR MATRIX OF MESANGIAL CELLS

Confluent mesangial cells were labelled with L-[2,3,4,5-3H] proline (Amersham), 100μCi/ml, overnight. Next day the extracellular matrix was isolated and 360μg/ml of matrix protein was run on an SDS gel.

GROWTH FACTORS USED

rIL-1 (Boehringer) and PMA (Boehringer).

CELL LINES

Mes (Human glomerular mesangial cells), BeWo (Choriocarcinoma, Human, Cancer Research. 28: 1231: 1986), Hep G2 ((Human hepatocellular carcinoma: Nature. 282: 616, 1979), and BHK (Fibroblast, baby hamster kidney).

RESULTS

SDS GEL USING HIGH MOLECULAR WEIGHT MARKERS WITH MATRIX OF BeWo, MESANGIAL, Hep G2 AND BHK

Matrix from BeWo, mesangial, Hep G2,and BHK cell lines which had been stimulated with PMA was reduced and run on SDS gel. A common band was obtained in the matix of non-stimulated and stimulated BeWo, mesangial, Hep G2 and BHK of 67-70 kd, and another band of about 220 kd was obtained in the matrix of stimulated and non-stimulated cell lines. A protein of 110 kd was demonstrated in the matrix of stimulated BeWo and mesangial cells and a unique band of 150 kd was found in the matrix of stimulated mesangial and BeWo cells. The band of 67-7- kd was more dense in the matix from stimulated cells than from non-stimulated cells (Figyre 1.1).

PROLINE SDS GEL ANALYSIS OF MESANGIAL MATRIX

Three bands were obtained in matrix of stimulated mesangial cells with molecular weight of 85, 107 and 150 kd which were not present in the matrix of non-stimulated cells, whereas there were two bands of 55 and 97 kd obtained in the matrix of non-stimulated cells but not in the matrix of stimulated cells (Figure 1.2).

DISCUSSION

The results of SDS gel analysis showed a common band of 65-70 kd in the matrix of both stimulated and non-stimulated mesangial cells, while a unique band of 150 kd was obtained in the matrix of stimulated mesangial and BeWo cells only. these data indicate the effect that cytokines have on the expression of different proteins. purified IL-1 from LPS-stimulated rat peritoneal macrophages, stimulated mesangial cells to secrete gelatinase in a dose-dependent manner (Martin et al, 1986).

Cultured human mesangial cells produce a variety of extracellular matrix (ECM) proteins and secretion of some are increased by cytokines. Ischimura et al (1989) mentioned that collagen IV or laminin secreted by mesangial cells in the substratum accelerates production and secretion of extracellular matrix constituents in an autocrine fashion, and showed that extracellular deposition of collagen IV appeared followed by fibronectin and collagen III. Release of TGF during glomerular injury can mediate accumulation of proteoglycans in the extracellular matrix and predispose the kidney to glomerulonephritis Border et al, 1990). Deregulation production of IL-6 may also be involved in proliferative glomerulonephritis (Horri et al, 1989).

The study shwed that cytokines aletered the production and secretion of extracellular matrix proteins.

REFERENCES

-Horri Y, Atushi M, Iwano M, et al. Involvement of IL-6 in mesangial proliferative glomerulonephritis. J Immunol. 1989: 12: 3949-3955.

-Ischimura E, Sterzel B, Budde K and Kashgarian. Formation of extracellular matrix by cultured mesangial cells. Am J Pathol. 1989; 134: 843-855.

-Martin J, Lovett D H, Gemsa D, Sterzel R B and Davies M. Enhancement of glomerular mesangial cell neutral protienases secretion by macrophages: role of interleulin 1. J Immunol. 1986: 137 (2): 525-529.

-Mukhtar A. 1992. Study of glomerulonephritis using monoclonal antibodies against mesangial matrix. PhD thesis. University of Aberdeen. UK.

-Laemmle U K. Cleavage of stuctural protein during the assembly of the head of the bacteriophage T4. Nature. 1970; 227: 680-685.

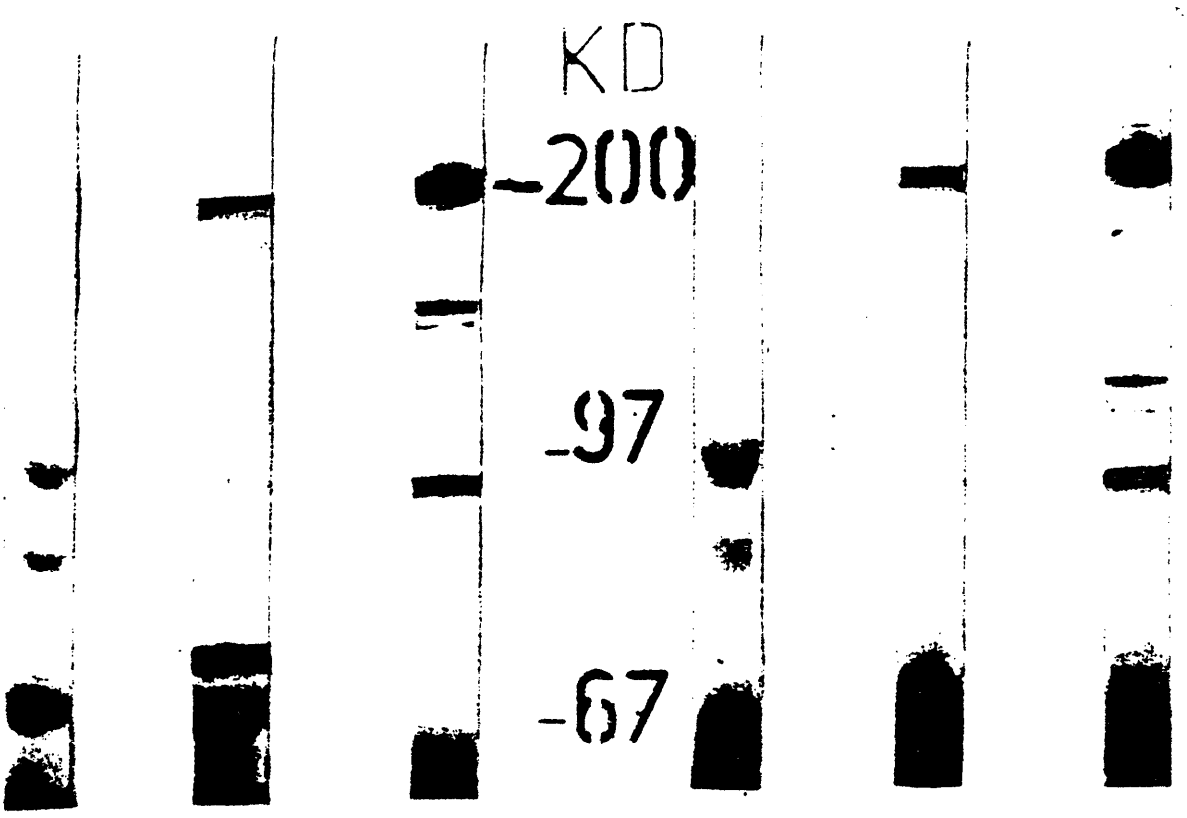


Figure 4.1 SDS gel (7.5%) of matrix of mesangial cells from right to left: cells stimulated with IL-1 (reducing buffer), non-stimulated cells (reducing buffer), standard high molecular weight (reducing buffer), cells stimulated with IL-1 (non-reducing buffer), non-stimulated cells (non-reducing buffer) and standard high molecular weght.

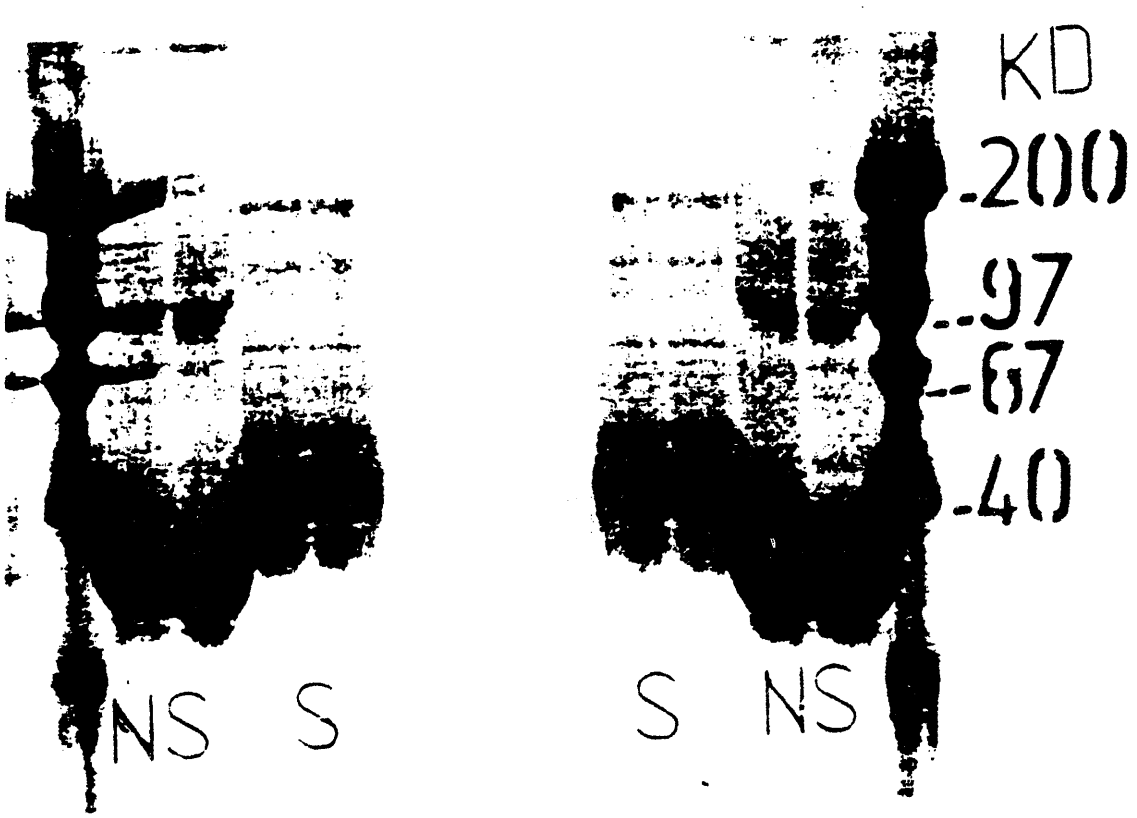


Figure 1.2 SDS gel of proline labelled matrix of non-stimulated and stimulated mesangial cells.

TRANSPLANTATION IMMUNOLOGY

SEX lINKED HLA -A AND -B ALLELES AMONG BAHRAINIS

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ارتباط الجنس مع جينات وراثة الاعضاء

تبين هذه الدراسة بوجود ارتباط بعض انواع موروثات زراعة الاعضاء مع الذكور والاناث ، ولقد تبين بان اكثر الانواع انتشارا هى : ١١ ، ٢١ ، ٩١ ، ب١١ ، ب٢١ ، ب٧ ، ب٨ ، ب٢ب٥ ، ب١٣ ، ج١ ، ج٢ ، ج٣ ، ج٤ ، و ج٦ .

تبين بان الموروثات الاتية االكثر انتشارا لدى الذكور : ب٥ ، ب١٨ ، وب٥٤ ، اما الموروثات الاتية فهى اكثر انتشارا لدى الاناث : ب٢ ، ب١٤ ، ب ٤ ، وب٤٤ ، وليس هناك تفسير علمى لذلك .

ABSTRACT

This study shows that there are some sex-linked HLA -A and -B antigens amonsgt Bahranis males and females. The study showed that HLA antigens, which shows Bahrainis with the following HLA of high frequencies: A2, A1, A19, A9 A10, A11, B12, B7, B8, B2, B5, B13, Cw4, Cw3, Cw1, Cw2, and Cw6. It was shown that the following HLA -B antigens were significantly more in females than males such as B5, B18 and B54, while others such as B2, B14, B4 and B44 were more in males than females. There is no explanation for this mechanism .

INTRODUCTION

The terms "tissue typing" and HLA typing are interchangeable, however many tissue antigens are encoded outwith the HLA. There are two classes of HLA molecules. Class I (44kd glycopeptides) molecules,and Class II molecules (34Kd a chain ond 29Kd chain) are the HLA-DR, DG and DP gene products and are restricted to B cells. HLA has many uses, from matching donor and recipient for organ and bone transplants, to studying genetic susceptibility to certain autoimmune diseases. Since the function of the HLA antigens is to initiate and regulate immune, HLA phenotyping (is identification of specific HLA antigen expression) is very important in conditions such as Arthritis. HLA typing , due to its extreme polymorphism, is used in investigation of paternity disputes and is a legally accepted method of serological discrimination.

MATERIALS AND METHODS

Six hundred and five blood specimens were collected (303 males and 302 females) from blood donors, students and staff College of Health Sciences and Arabian Gulf University, and Staff of Ministry Health and BDF Hospital. All were Bahraini nationals.

TYPING METHOD

1. Remove the appropriate typing trays from the -40 C freezer and allow to thaw at room temerature.
2. Add 1μl cell suspension to each well of the appropriate typing trays, using the "shooting" method.

NB - "Shooting method uses a Hamilton syringe with a square cut needle. The tip of the needle is held just above the well, clear of the oil and as the button on the dispenser is pressed the 1μl drop "shoots" into the well, this method prevents carryover and the need to wipe the tip of the needle between the wells.

4. Incubate in 22°C incubator for 30 minutes.
5. Add 5μl complement /PI mixture to each well using "soft drop" technique.

NB - The "Soft drop" method uses a Hamilton multidespenser (six

syringes operated simultaneously). The tips of the needles are heldjust above the wells, clear of the oil, the button is pressed gently and the drop of complement or ink is placed onto the oil so that it sinks slowly down through the well to mix thus avoiding distirbing the cells on the bottom of the wells.

rotocols

rticular

6. Incubate at 22°C for 45 minutes (Dynabeads) or 60 minutes (Ficoll -Hypaque).

7. Stop reaction after appropriate incubation by addition of 5μl EDTA/Higgins Ink solution to each well, using "soft drop" technique as in 6.

8. Place plates at 4°C for 1 minimum of 15 minutes before reading.

9. Read plates on the inverted fluorescent microscope, using blue excitation filter.

10.Results:

Live cells stain: Green

Dead cells stain: Red

% Dead cells (Red) are scored according to the W.H.O. convention:

Score	Cell Death
X	Not tested
O/Blank	Negative
2	11%-20%

RESULTS

The study of gene frequencies of HLA -A, antigens showed that A2 locus was the most common, with the highest percentage of the total sample (36.6%), and A1 as a the second highest percentage of the total (17.8). The other important HLA -A antigens were as follows: A19 (8%), A9 (7.3%), A10 (5.2%), A24 (2.1%), A 29 (2.3%), A23 (1.7%) and A26 (1.9%).The study of gene frequencies of HLA -B antigens showed that B12 was the most common with the highest percentage of the total sample (23.3%), and B7 as a second highest percentage (15.3%). The other important HLA -B antigens were as follows: B8 (9.6%), B51 (6.9%), B5 (65), B15 (5.6%), B13 (3%), B22 (2.1%), B40 (2%), B21 (1.9%), B14 (1.6%), B27 (1.9%), and B44 (1.1%).

The study of gene frequencies of HLA -C antigens showed that Cw4 was the most common, with the highest percentage oh the total sample (22.4%), and Cw4 (21%) as a second highest HLA -C antigens. The other important HLA -C antigens were as follows: Cw1 (10.4%), Cw2 (9.7%), Cw6 (9.7), Cw5 (1.6%), Cw8 (1.5), Cw7 (1.2%), Cw9 (1.9), and Cw10 (0.4%).

There was some differences of HLA -A and -B antigens amongst males and females such as A11, it was 6.3% for males of the total HLA -A of males, and 3.4% for the females of the total HLA -A amongst the

females. The other HLA -A antigens with some differencebetween males and females were as follows: A2 (39%) for males and 42% foe females, A9 (7.9%) for males and 6.6% for females, A19 (8.6%) for males and 7.4% for females, A28 (3%) for males and 2.3% for females.

It was shown that there are some differences between HLA -B antigens for males and females. The following HLA -B antigens were higher frequency amongst females than males: B5 (7.8%) for females and 4% for males, B18 (0.7) for females and 0.3% for males, B62 (0.4%) for females and 0.2% for males, B54 (2.2%) for females and 0.5% for males, B35 (5.5%) for females and 4.3% for males, and B27 (1.8%) for females and 1.3% for males. While the following HLA -B antigens frequecies were higher in males than females: B2 (1.2%) for males and 0.3% for females, B14 (2.3%) for males and O.8% for females, B4 (2.6%) for males and 1.3% for females, B44 (1.4%) for males and 0.6% for females, B54 (1%) for males and 0.2% for females, B47 (0.8%) for males and 0.5% for females, B5 (7/8%) for males and 6% for females, B57 (0.3%) for males and 0% for females, and B58 (0.5%) for males and 0.2% females.

DISCUSSION

This study showed that there are some differences for HLA -A and -B antigens between Bahrainis males and females. Some were significantly more in females than males such as B%, B18 and B54, while others were significantly more in males than females such as: B2, B14, B4, and B44. There is no explanation for this, since a totally random distribution of the antigens should occur. The sample size (605 persons) is not small for the first survey of HLA -A and -B amongst Bahranis, and the selection of the population sample was random. The differences amongst diffrerent ethnic groups is known. In Caucasian population the haplotype combination A1-B8, A2-B12 and A3-B7 are all much commoner than would be expected from ranodom assortment. There may be selective advantages for individuals having certain haplotypes, but a totally convincing explanation for the mechanism is still waited (Williamson A R & Turner M W, 1987).

Bahrain consist of multicultural community, and played a significant role in the old civilization, where many communities settled and migrated and this could play role in some HLA differences found amongst males and females.

REFERENCES

Williamson AR and Turner MW.1987. Essential Immunology. Blackwell Scientific Publication; 215-255.

Gene Frequencies of HLA-A Locus Amongst Males and Females

A Locus	Male	Female
1	109	106
2	236	255
3	4	4
4	1	0
9	48	40
10	32	31
11	38	21
12	1	3
17	0	1
19	52	45
20	1	0
21	0	1
22	0	1
23	10	10
24	14	12
25	3	3
26	11	12
28	18	14
29	0	3
30	15	13
31	4	4
32	0	4
33	0	1
34	0	2
36	3	5
39	1	0

TABLE 1

TABLE 2

Gene Frequencies of HLA-B Locus Amongst Males and Females

B Locus	Male	Female
1	2	2
2	7	2
5	25	47
6	1	0
7	93	92
8	60	56
9	0	1
10	3	0
11	2	1
12	168	187
13	19	17
14	14	5
15	33	35
16	2	4
17	4	5
18	2	4
21	12	11
22	13	12
27	8	11
29	1	0
31	0	1
35	26	33
37	0	4
38	1	0
39	4	5
40	16	8
41	0	1
42	5	5
43	2	5
47	0	1
66	2	0
68	1	2
69	0	4
74	0	1

TABLE 3

Gene Frequencies of HLA-B Locus Amongst Males and Females

B Locus	Male	Female
44	9	4
45	6	1
46	1	0
47	5	3
48	0	1
51	47	36
53	1	0
54	3	13
55	5	4
57	2	0
58	3	1
59	1	1
62	1	2
71	1	0

TABLE 4

Gene Frequencies of HLA-C Locus Amongst Males and Females*

B Locus	Male %	Female %
1	5.3	5.1
2	4.7	5.0
3	11.1	9.9
4	11.2	11.3
5	.7	.8
6	4.8	4.9
7	.6	.6
8	.8	.7
9	.5	.6
10	.3	.1
Blank	11.0	9.0

HLA -A, -B and -C ALLELES AMONG BAHRAINIS

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موروثات زراعة الاعضاء لدى المواطنين البحرينيين

هذه اول دراسة لموروثات زراعة الاعضاء لدى الموطنين البحرينيين ، حيث تساعد للتخطيط لمركز زراعة الاعضاء مستقبلا ، ٣٠٣ من الذكور ، و ٣٠٢ من الاناث تم اجراء الدراسة عليهم .

ولقد تبين ارتباط البحرين بالشرق الاوسط من خلال الموروثات الشائعة الاتية : ١١، ٢١، ٩١١ ، ١٠١ ، ١١١ ، ١٩١ ،ب١٢ ، ب٥ ، ب٢ ، ب١٣ ، ج١، ج٢ ، ج٣ ، ج٤ ، ج٦ .

ABSTRACT

This is the first study of tissue typing (HLA) Class I amongst Bahrainis, which will indicate the HLA -A, -B, and -C amongst Bahrainis and will help in the planning of Transplantation Centre. Six hundred and five Bahrainis were included in the study (303 males: 302 females). The complement-dependent microcytotoxicity CDC was used, which is the aknowledged International Standard Test procedure. The study showed that there are unique HLA antigens, which shows Bahrainis relation to the Middle East, with the following characteristics of high frequencies: A2, A1, A19, A9 A10, A11, B12, B7, B8, B2, B5, B13, Cw4, Cw3, Cw1, Cw2, and Cw6. Some of the antigens were shared with other countries.

INTRODUCTION

The terms "tissue typing" and HLA typing are interchangeable, however many tissue antigens are encoded outwith the HLA. There are two classes of HLA molecules. Class I (44kd glycopeptides) molecules,and Class II molecules (34Kd a chain ond 29Kd chain) are the HLA-DR, DG and DP gene products and are restricted to B cells. HLA has many uses, from matching donor and recipient for organ and bone transplants, to studying genetic susceptibility to certain autoimmune diseases. Since the function of the HLA antigens is to initiate and regulate immune, HLA phenotyping (is identification of specific HLA antigen expression) is very important in conditions such as Arthritis. HLA typing , due to its extreme polymorphism, is used in investigation of paternity disputes and is a legally accepted method of serological discrimination.

MATERIALS AND METHODS

Six hundred and five blood specimens were collected (303 males and 302 females) from blood donors, students and staff College of Health Sciences and Arabian Gulf University, and Staff of Ministry Health and BDF Hospital. All were Bahraini nationals.

MATERIALS

1. Phosphate buffered saline (PBS).
2. RPMI 1640-Protein Fractionation Cetre, Ellen's Glen Road, Edinburgh. Supplied as 500ml sterile volume, without L-Glutamine, with 20mM Hepes and 1mM Pyruvate. Store at 4°C.
3. Pencilliin/Streptomycin, 5000iu/ml each -Flow Laboratories Ltd., Harefield Road, Rickmansworth, Herts WD3 1PQ. Code 16-700 49, 100ml volume. Bulk stock stored in -4°C in Tissue Typing Lab. For use add 2ml/100ml medium to give a final concentration of 100 units each per ml.
4. Pooled AB serum. Recalcified plasma from group AB donors. Tested for the presence of cytotoxic antibodies prior to use. Stored at -40C (Cryo-King U/R Freezer no1).

5. Rabbit Complement -Freeze dried rabbit serum (1ml, 2ml & 5ml vials), stored at 4°C. Reconstitute with 1ml, 2ml and 5ml sterile distilled water prior to use. All complement is checked prior to acceptance for use.

6. Carboxy-Fluorescien Diacetate (C-FDA) - Cambridge BioScience, Newton House, 42 Devonshire Road, Cabridge CB1 2BL. Calbiochem 216275, 50mg. A stock solution of 10mg in 2 ml Analar acetone is made and stored at 4°C. A working solution of 40 μl solution in 5ml PBS is prepared on day of use, stored at 4°C and remaining stock solution discarded at end of day.

7. Pripodium iodide (PI)- Sigma Chemical Co., Fancy Road, Poole Dorset BH17 7NH. P-4170, 250mg. Astock solution of 2mg/ml in PBS is made and stored at 4°C. 20 l of stock solution is added per 1ml rabbit complement just prior to use.

8. EDTA/PBS (0.5%) - Sigma Chemical C0. Ltd. Fancy Road, Poole, Dorset BH17 7NH. ED2SS Disodium EDTA 500mg. Weigh out 5g Na2 EDTA. Add to 1 litre PBS. Store at 4°C.

9. Higgins Unk - Miller's Graphics, George St. Aberdeen. Faber-Castell No 44314, supplied as 74ml neat ink. Add 4ml ink to 96ml of 0.5% Na2 EDATA?PBS. Store at 4°C.

10.Ficoll-Hypaque -100ml or 500ml volumes, sterile density 1.077 + 0.001 at 22°C. Store at 4°C protected from light. Remove quantities

required using a sterile disposable syringe.

11.Dynabeads - Dynal (UK) Ltd. Station House, 26 Grove St., New Ferry, Wirral. Supplied in 5ml vulomes of either HLA Class I (code 21002) or HLA Class II (code 21004) stored at 4°C, ready for use. (Bulk stored at -20 C).

12.HLA Typing trays (Teraski Plates) - Greiner Labortechnik., Station Road, Cam, Dursley, Glos. GL11 5NS. Code 654180, 72 well typing trays.

13.Paraffin Oil - Pharmacy., ARI. 250ml volume, light liquid paraffin.

14.Heparin Vials- Monoparin 5000iu/ampoule, 2 ampoules (10,000iu in 0.4ml), are added to 9.6ml of RPMI 1640 to give a concentration of 1000iu/ml. Add 400ml of Heparin to a labelled Universal container using sterile technique in the alminar flow cabinet. Store vials upright at 4°C.

15.Sterlin universal containers - Sterlin code 128B, sterile, 30ml with label.

16.Sterlin conical centrifuge tube - Sterlin code 144AS, sterile.

17.Pastettes, 1ml and 3ml - Alpha Laboratories Ltd., 40 Parham Drive, Eastleigh, Hants. Code LW4040 (1ml), LW4111 (3ml).

18.Chloros, 10% in water. Concentrate kept in washup.

19.Disposable syringes and needles.

20."Coolit" insulated ice bucket, crushed ice, test tube racks, counting chambers, magnets for Dynabeads, marker pens, all keptin lab.

21.Centrifuge, Centra 4B -Damon IEC.

22.Microscope, Olympus BH-2 binocular with fluorescent attachment.

23.Microscpoe, Olympus IMT-2 inverted with fluorescent attachment.

PREPARATION OF LYMPHOCYTE SUSPENSION

Class I typing - ie HLA -A, -B, and -C can be performed directly on lymphocytes extracted from peripheral blood. Class I lymphocytes was extracted using Class I Dynabeads. Where an urgent result is required Class I lymphocytes can be extracted from whole blood.

TYPING METHOD

1. Remove the appropriate typing trays from the -40 C freezer and allow to thaw at room temerature.

2. Add 1μl cell suspension to each well of the appropriate typing trays, using the "shooting" method.

NB - "Shooting method uses a Hamilton syringe with a square cut needle. The tip of the needle is held just above the well, clear of

the oil and as the button on the dispenser is pressed the 1μl drop "shoots" into the well, this method prevents carryover and the need to wipe the tip of the needle between the wells.

4. Incubate in 22°C incubator for 30 minutes.

5. Add 5μl complement /PI mixture to each well using "soft drop" technique.

NB - The "Soft drop" method uses a Hamilton multidespenser (six syringes operated simultaneously). The tips of the needles are heldjust above the wells, clear of the oil, the button is pressed gently and the drop of complement or ink is placed onto the oil so that it sinks slowly down through the well to mix thus avoiding distirbing the cells on the bottom of the wells.

6. Incubate at 22°C for 45 minutes (Dynabeads) or 60 minutes (Ficoll -Hypaque).

7. Stop reaction after appropriate incubation by addition of 5μl EDTA/Higgins Ink solution to each well, using "soft drop" technique as in 6.

8. Place plates at 4°C for 1 minimum of 15 minutes before reading.

9. Read plates on the inverted fluorescent microscope, using blue excitation filter.

10.Results:

Live cells stain: Green

Dead cells stain: Red

% Dead cells (Red) are scored according to the W.H.O. convention:

Score	Cell Death
X	Not tested
O/Blank	Negative
2	11%-20%
4	21%-40%
6	41%-80%
8	81%-100%

11. Results are recorded on the appropriate Typing Tray protocols and dealt with in accordance with the procedure for the particular typing request.

RESULTS

Six hundred and five Bahrainis were involved in the project. Three hundred and three persons were males and three hundred and two were females. The age ranged between 17-5 years old. The samples were colected as follows:

162 persons: Blood Bonk/ Salmaniya Medical Centre & BDF Hospital.

351 persons: Students/ College of Health Sciences, Bahrain University.

54 persons: Staff/ Ministry of Health, BDF Hospital & Bahrain Unversity.

38 persons: Others.

The study of gene frequencies of HLA -A, antigens showed that A2 locus was the most common, with the highest percentage of the total sample (36.6%), and A1 as a the second highest percentage of the total (17.8). The other important HLA -A antigens were as follows: A19 (8%), A9 (7.3%), A10 (5.2%), A24 (2.1%), A 29 (2.3%), A23 (1.7%) and A26 (1.9%).The study of gene frequencies of HLA -B antigens showed that B12 was the most common with the highest percentage of the total sample (23.3%), and B7 as a second highest

percentage (15.3%). The other important HLA -B antigens were as follows: B8 (9.6%), B51 (6.9%), B5 (65), B15 (5.6%), B13 (3%), B22 (2.1%), B40 (2%), B21 (1.9%), B14 (1.6%), B27 (1.9%), and B44 (1.1%).

The study of gene frequencies of HLA -C antigens showed that Cw4 was the most common, with the highest percentage oh the total sample (22.4%), and Cw4 (21%) as a second highest HLA -C antigens. The other important HLA -C antigens were as follows: Cw1 (10.4%), Cw2 (9.7%), Cw6 (9.7), Cw5 (1.6%), Cw8 (1.5), Cw7 (1.2%), Cw9 (1.9), and Cw10 (0.4%).

DISCUSSION

This is the first study of HLA -A, -B and -C gene freqencies amongst Bahrainis. The study showed that the most common HLA -A gene freqencies was A2 with the 36.6% of the total sample size and A1 with the second highest (17.8%) of the total sample size. This is more than A2 antigen amongst British Caucasian (29.1%), North American Caucasian (28%), North American Negroes (16.3%), Japanese (25.3%) (Williamson A R & Turner A R, 1987), and less than than A2 in a survey of pregnant women Caucasian (46.2%) and A2 amongst Orientals (44.6%) (Yoshikiki K, et al 1986) and more than A2 among Zambians (14.9%), Malays (15%), European Caucasoids (27%) and less than A2 amongst Amerindians of mixed origin, predominantly North Americans (45.3%) (Joysey V C, & Wolf E, 1978). A1 gene frequency was higher than A1 gene frequency among European Caucasoids (15%), Zambians (2.3%), Malays (2.6%) and Amerindians of mixed origin, predominantly North Americans (45.3%) (Joysey V C & Wolf E, 1987). A1 gene frequency was higher than A1 frequency among European Caucasoids (15%), Zambians (2.3%), Malays (2.6%) and Amerindians of mixed origin, predominantly North American (2.5%) (Joysey C C& Wolf E), and less than A1 amongst British Caucasian (1601%), North

American Negroes (8.1%) and Japanese (8.1%) (Wiiliamson A R & Turner A R, 1987).A9 gene frequency amongst Bahrainis was 7.3% of the total HLA -A gene frequency. It is less than A9 gene freqency amongst Malays 40.1%), Japanese (37.2%), Zambians (10.7%) and European Caucasoids (11.3%) (Joysey V C & Wolfe E), and less than A9 gene frequency amongst British Caucasoids (9.5%), North American Caucasian (9.2%), North American Negroes (16.3%) and Japanese (25.3%) (Williamson A R & Turner A R, 1987).A10 gene frequency amongst Bahrainis was 5.2% which is higher than A10 amongst European Caucasoids (6.8%), Zambians (3.7%), Malays (1.1%), Amerindians of mixed origin, predominantly North American (16%), but less than A10 in Japanese (12.7%) (Joysey V C & WolF E, 1978), and it was more than A10 amongst British Caucasian (4.4%), North American Caucasian (5.9%), and less than less than amongst North American Negroes (2.7%) (Williamson A R & Turner A R, 1987). A11 gene frequency was expressed in 4.1% of total sample size. It was 5.1% amongst European Caucasoids, 0% amongst Zambians, 6.7% amongst Japanese, 16.4% amongst Malays (Joysey V C & Wolf E, 1978). A28 gene frequency was expressed in 0.2% of total HLA -A sample. It was less than reported in British Caucasoids (4%), 4.2% in North American Caucasian, 5.8% in North American Negroes and 0% in Japanese (Williamson A R & Turner A R, 1978). Other HLA -A genes

were first time reported in Gulf area after WHO 1991 Workshop such as A66, A68, A69, A74.

The study of B12 gene frequency showed that it was expressed in 23.3% of Bahrainis. It was more than B12 in European caucasoids (16.6%), 11.7% in Zambians, 6.5% in Japanese, 3.3% in Malays (Joysey V C & Wolf E, 1978), British Caucasian (20.8%), North American Caucasoids (13.8%), and North American Negroes (Williamson A R & Turner A R, 1987). B7 gene freqency was expressed in 15.3% of Bahrainis. It was more than B7 in European Caucasoids 10.4%, Zambians (8.6%), Japanese (7.1%), Malays (5.3%) (Joysey V C & Wolf E, 1978), and British Caucasian (12.5%), North American Caucasian (10.5%), North American Negroes (12.6%) and Japanese 7.1% (Williamson A R & Turner A R, 1987). B8 gene frequency was expressed in 9.6% of Bahrainis, more than European Caucasoids (9.2%), Zambians (3.7%), Japanese (0.3%) and Malays (0%) (Joysey V C & Wolf E, 1978), but less than British Caucasian (13.4%) and North American Caucasian (10.4%) (Williamson A R & Turner A R, 1987). B5 was expressed in Bahrainis (6%) less than japanese (20.9%), B15 was expressed in 5.6% of Bahrainis less than reported in Malays (29%) (Joysey V C & Wolf E) and Amerindians of mixed origin, predominantly North American (13.7%). B51 was expressed in 6.9% of Bahrainis, less than reported in Oriental pregnant women

(25%) (Yashiki K, et al 1986).

Other HLA -B antigen were first time reported in Gulf and in a survey study such as B62, B71 and B77. The study of HLA -C antigens frequency showed that Cw4 was expressed in 22.4% of total sample size, which is less than it's expression in European Caucasoids (12.6%), Zambians (15.9%), Japanese (4.3%) and more than its expression amongst Amerindians of mixed origin, predominantly North American (23.4%) (Joysey V C & Wolf E, 1978).Cw3 was expressed in 21% of Bahrainis which is less than reported in European Caucasoids, Zambians, Japanese, Malays (Joysey V C & Wolf E, 1978), and North American Negroes (Williamson A R & Turner A R, 1987). Cw1 amongst Bahrainis was expressed more than reported in Eurpean Caucasoids, Zambians, Malays, but less than in Japanese (Joysey V C & Wolf E, 1978). Cw6 amongst Bahrainis was less than reported in European caucasoids, but more than reported in Malays, Zambians and Japanese (Joysey V C & Wolf E, 1978).

This study shows that there are unique HLA genes amongst Bahrainis, with the following genetic characteristics: A2, A1, A19, A9, A10, A11, B12, B7, B8, B2, B5, B13, Cw4, Cw3, Cw2, and Cw6. This is due to that Bahrain was in the centre of old civilzations.

REFERENCES

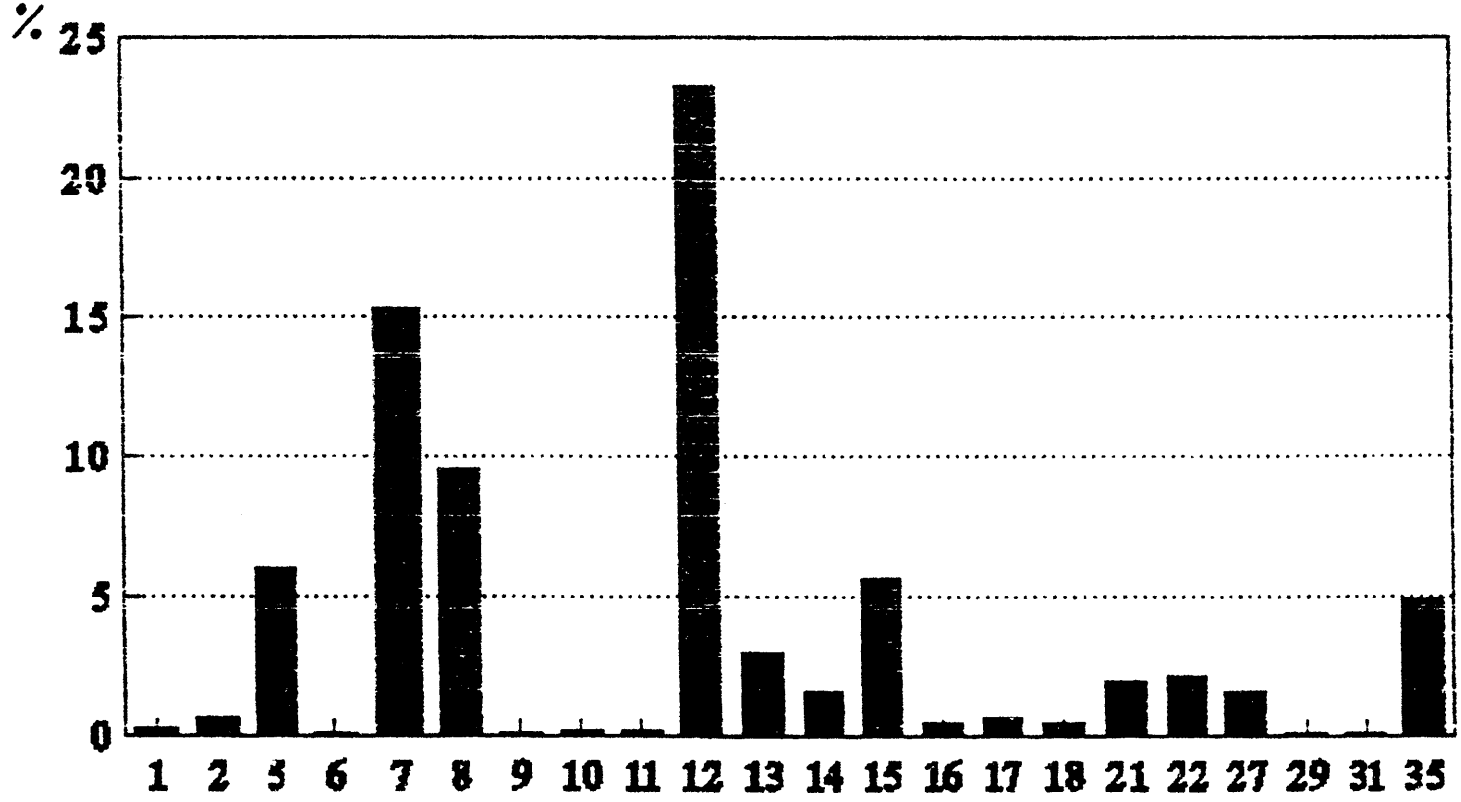
-Joysey V C & Wolf E. 1978. HLA -A, -B, and -C antigens their Serology and reaction. Br Med Bull., 34, 217.

-Willuamson A R & Turner A R. 1987. Essential Immunology. Blackwell Scientific Publication; 215-254.

-Yoshiki K, Terasaki P I, Wakisaka A, Park M S and Mickey M R. 1986. Public determinants of HLA indicated by Pregnancy Antibodies. Transplantation; 41 (2): 253-259.

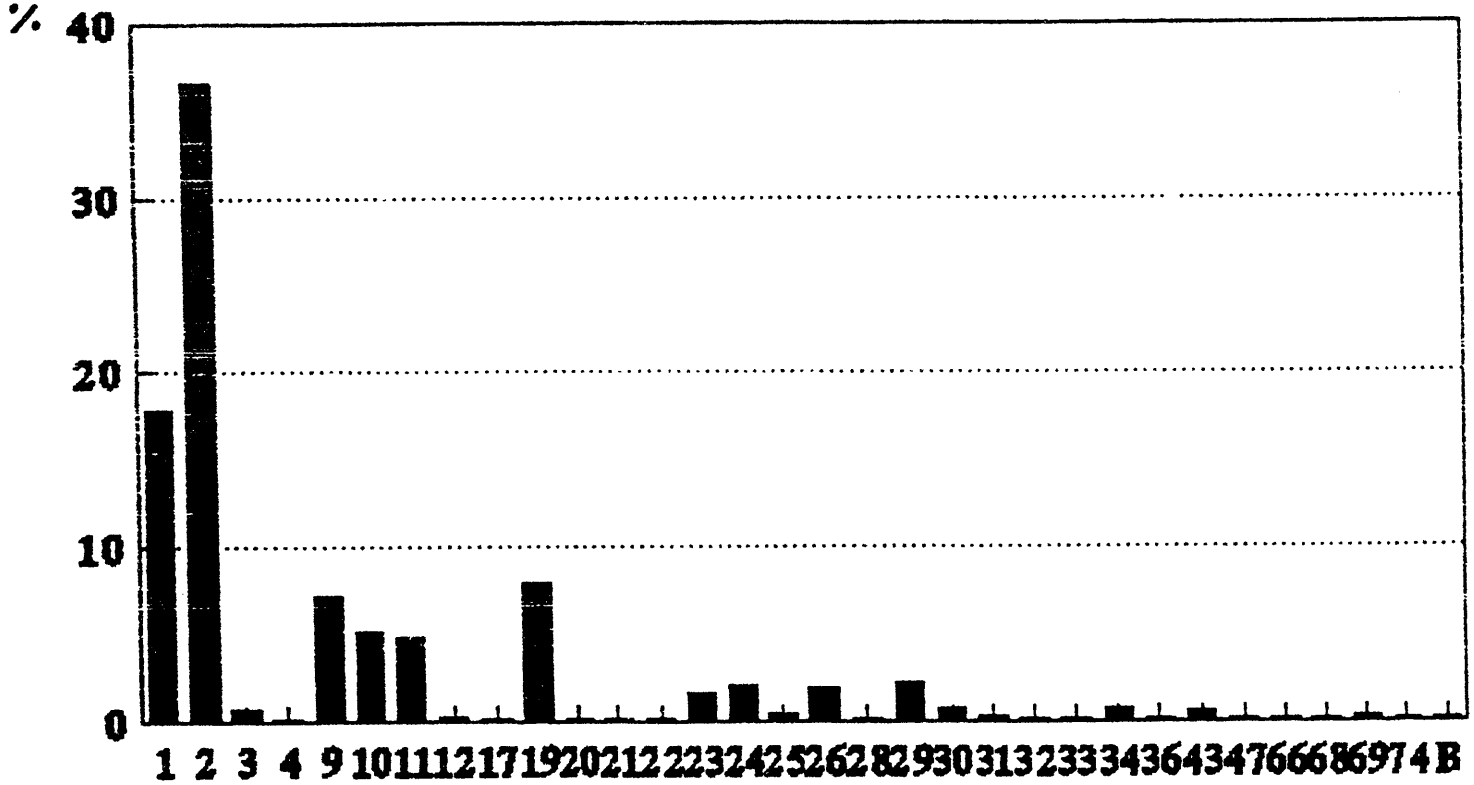
Gene Frequencies of HLA Antigens

TABLE 2 Percentage of B Locus



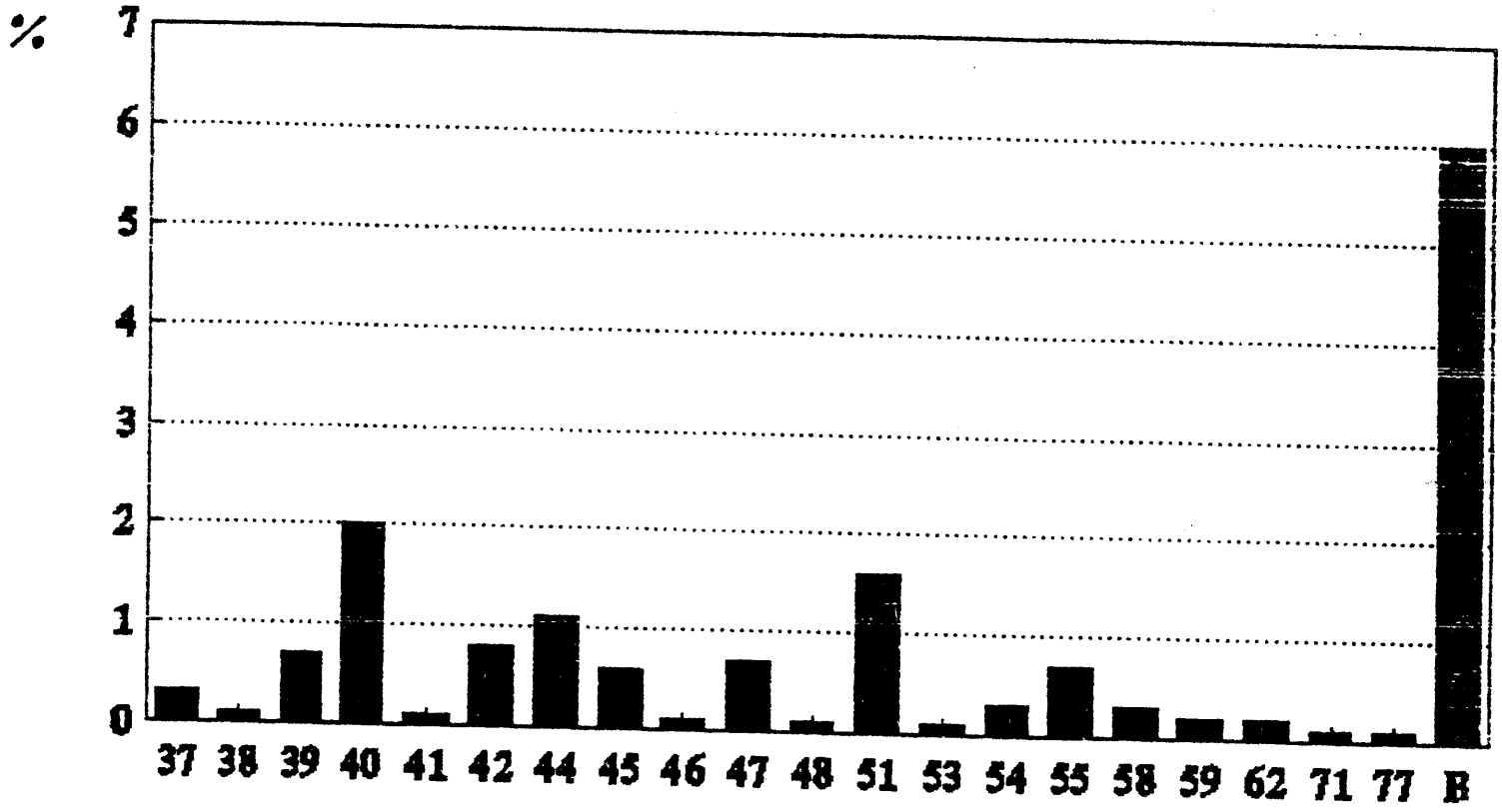
Gene Frequency of HLA Antigens

TABLE 1 Percentage of A locus



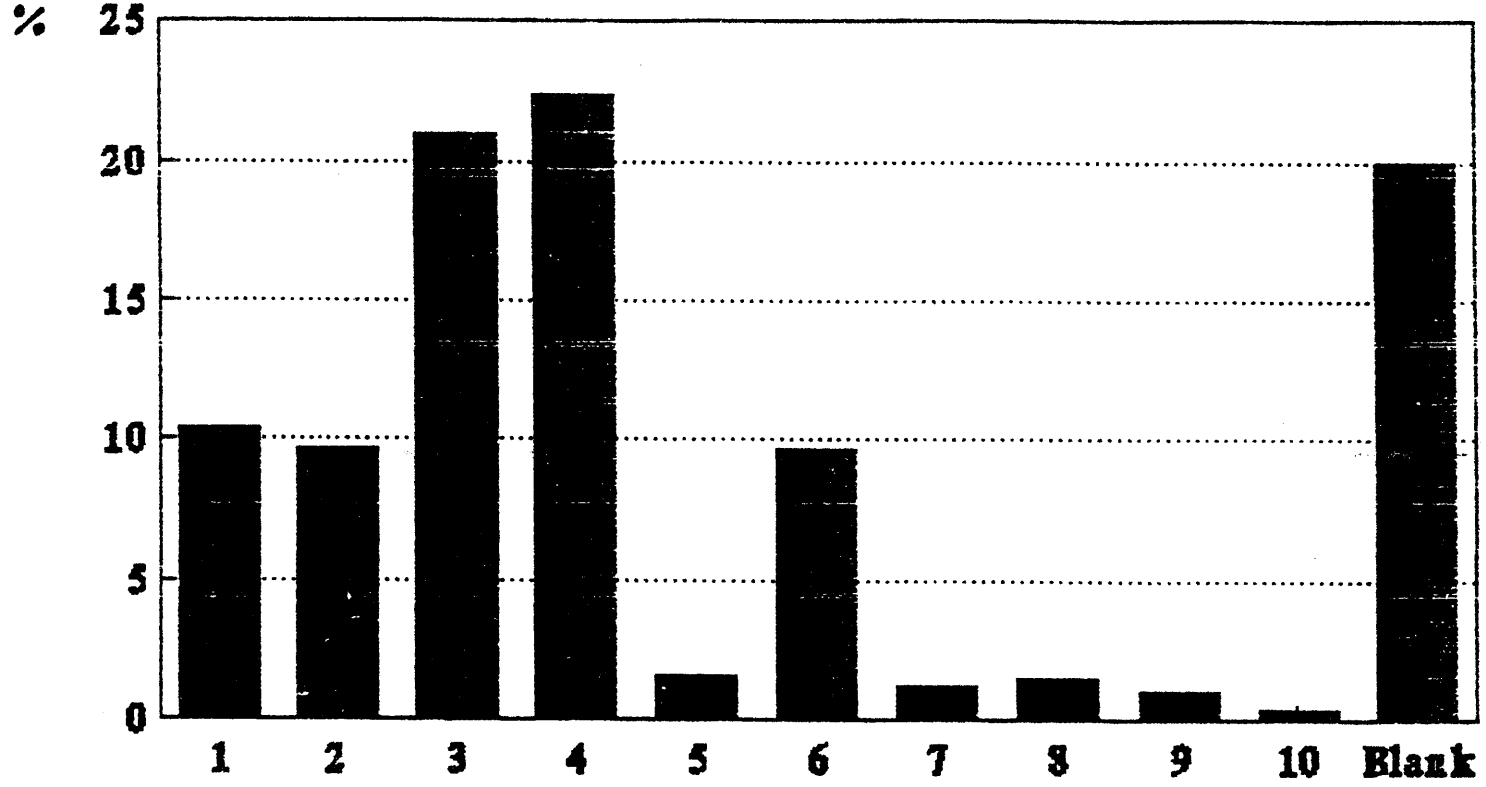
B: Blank

TABLE 3 Percentage of B Locus



B: Blank

TABLE 4 Percentage of C Locus



A, B, AB & O BLOOD GROUPS ASSOCIATION WITH HLA -A, -B and -C ALLELES AMONG BAHRAINIS

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ارتباط فصائل الدم مع موروثات زراعة اللاعضاء لدى البحرينيين

هذه اول دراسة لارتباط موروثات زراعة الاعضاء مع فصائل الدم لدى المواطنين البحرينيين ، حيث تم الدراسة على ٣٠٣ من الذكور و ٣٠٢ من الاناث .

لقد تبين ارتباط البحرين بالشرق الاوسط وكذلك ارتباط بعض الموروثات بالفصائل الدم المختلفة، وهذه الدراسة مهمة لعملية نقل الدم وزراعة الاعضاء .

ABSTRACT

This is the first study of tissue typing (HLA) Class I amongst Bahrainis, which will indicate the HLA -A, -B, and -C amongst Bahrainis in correlation with A, B, AB and O blood groups. Six hundred and five Bahrainis were included in the study (303 males: 302 females). The complement-dependent microcytotoxicity CDC was used. The study showed that there are unique HLA antigens, which shows Bahrainis relation to the Middle East, with the following characteristics of high frequencies: A2, A1, A19, A9 A10, A11, B12, B7, B8, B2, B5, B13, Cw4, Cw3, Cw1, Cw2, and Cw6. Some of the antigens were shared with other countries. The results showed that some HLA -A, -B and -C antigens are linked to certain A, B, AB and O blood groups, which is important for blood transfusion and organ transplantation.

INTRODUCTION

HLA (Human Leucocytes Antigen) gene comples is located on chromosome 6 within the area called the Major Histocompatibilty Complex (MHC) and is a series of linked genes coding for many proteins involved in forgein antigen recognition and responses. The terms "tissue typing" and HLA typing are interchangeable, however many tissue antigens are encoded outwith the HLA. There are two classes of HLA molecules, Class I (44kd glycopeptides) molecules and Class II molecules (34Kd a chain ond 29Kd chain)

HLA has many uses, from matching donor and recipient for organ and bone transplants, to studying genetic susceptibility to certain autoimmune diseases. Since the function of the HLA antigens is to initiate and regulate immune, HLA phenotyping (is identification of specific HLA antigen expression) is very important in conditions such as Arthritis. HLA typing , due to its extreme polymorphism, is used in investigation of paternity disputes and is a legally accepted method of serological discrimination.The "complement-dependent microcytotoxicity" (CDC) or " lymphocytotoxic" test is the aknowledged International Standard Test procedure to both type unknown lymphocytes and detect anti-HLA cytotoxic antibodies.

MATERIALS AND METHODS

Six hundred and five blood specimens were collected (303 males and 302 females). All were Bahraini nationals.

TYPING METHOD

1. Remove the appropriate typing trays from the -40 C freezer and allow to thaw at room temerature.

2. Add 1μl cell suspension to each well of the appropriate typing trays, using the "shooting" method.

NB - "Shooting method uses a Hamilton syringe with a square cut needle. The tip of the needle is held just above the well, clear of the oil and as the button on the dispenser is pressed the 1μl drop "shoots" into the well, this method prevents carryover and the need to wipe the tip of the needle between the wells.

4. Incubate in 22°C incubator for 30 minutes.

5. Add 5μl complement /PI mixture to each well using "soft drop" technique.

NB - The "Soft drop" method uses a Hamilton multidespenser (six syringes operated simultaneously). The tips of the needles are heldjust above the wells, clear of the oil, the button is pressed gently and the drop of complement or ink is placed onto the oil so that it sinks slowly down through the well to mix thus avoiding distirbing the cells on the bottom of the wells.

6. Incubate at 22°C for 45 minutes (Dynabeads) or 60 minutes (Ficoll -Hypaque).

7. Stop reaction after appropriate incubation by addition of 5μl EDTA/Higgins Ink solution to each well, using "soft drop" technique as in 6.

8. Place plates at 4°C for 1 minimum of 15 minutes before reading.

9. Read plates on the inverted fluorescent microscope, using blue excitation filter.

10.Results:

Live cells stain: Green

Dead cells stain: Red

% Dead cells (Red) are scored according to the W.H.O. convention:

Score	Cell Death
X	Not tested
O/Blank	Negative
2	11%-20%
4	21%-40%
6	41%-80%
8	81%-100%

11. Results are recorded on the appropriate Typing Tray protocols and dealt with in accordance with the procedure for the particular typing request.

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11. Results are recorded on the appropriate Typing Tray protocols and dealt with in accordance with the procedure for the particular typing request.

DISCUSSION

It was shown that the most common HLA -A, -B & -C antigens were: A1, A2, A9, A10, A11, A12, B2, B5, B7, B8, B12, B13, Cw2, Cw3, Cw4 and Cw6.

Several blood group antigen have been reported to be determined by or dependent on HLA-linked genes for expression. These include the Chido, Rodgers, and Bg red cell antigen sustem (Paul W E, 1989). This study shows that some HLA -A, -B and -C antigens are more linked to some A, B, AB and O blood groups. It was shown that blood group O is associated with the following HLA -B antigens: B16, B18, B2 and B4, with the highest with, where 83.3% of the total Bahrainis with B18 were of blood group O, whereas the blood group O composed of 46% of the total sample size.

It was shown that blood group A is associated with HLA -B1 antigen whereas 75% of the total B1 antigens where amongst blood group A. The study showed that blood group B is associated with HLA -B62, B57 and B58 whom 66.7% of the Bahrainis with HLA -B62 were of blood group B. Also it was shown that blood group AB is associated with HLA -B11 and C3 antigens, whom 66.7% of Bahrainis with B11 antigen were ob blood group AB.

The results shows that some HLA -A, -B and -C antigens are linked to certain A, B, AB and O blood groups, which is important for

blood transfusion and organ transplantation.

REFERENCES

Mukhtar A (1994). Transplantation HLA -A, -B and -C Alleles Amongst Bahrainis. College of Health Sciences, Bahrain.

Paul W E (1989). Fundamental Immunology. 2nd Edition. Raven Press, New York; 480-487.

RESULTS

Six hundred and five Bahrainis were involved in the project. Three hundred and three persons were males and three hundred and two were females. The age ranged between 17-5 years old.

The study of gene frequencies of HLA -A, antigens showed that A2 locus was the most common, with the highest percentage of the total sample (36.6%), and A1 as a the second highest percente (17.8). The other important HLA -A antigens were as follows: A19 (8%), A9 (7.3%), A10 (5.2%), A24 (2.1%), A 29 (2.3%), A23 (1.7%) and A26 (1.9%).The study of gene frequencies of HLA -B antigens showed that B12 was the most common with the highest percentage of the total sample (23.3%), and B7 as a second highest percentage (15.3%). The other important HLA -B antigens were as follows: B8 (9.6%), B51 (6.9%), B5 (65), B15 (5.6%), B13 (3%), B22 (2.1%), B40 (2%), B21 (1.9%), B14 (1.6%), B27 (1.9%), and B44 (1.1%).

The study of gene frequencies of HLA -C antigens showed that Cw4 was the most common, with the percentage the total sample (22.4%), and Cw4 (21%) as a second highest. The other important HLA -C antigens were as follows: Cw1 (10.4%), Cw2 (9.7%), Cw6 (9.7), Cw5 (1.6%), Cw8 (1.5), Cw7 (1.2%), Cw9 (1.9), and Cw10 (0.4%).

CYTOKINES

EFFECT OF CYTOKINES ON MESANGIAL EXTRACELLULAR MATRIX PROTEINS

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تاثير المواد المنشطة على بروتينات
وسادة خلايا الميزانجيال

خلايا الميزانجيال لموقعها المتميز ، القدرة على التقلص ، انتاج المواد المنشطة والتلاصق لها القدرة على احداث اصابات بالكلية .

هذه الدراسة تبين تاثيرالمواد المنشطة على انتاج بروتينات وسادة خلايا الميزانجيال المنشطة والغير المنشطة ، المواد المنشطة التى درست هى : بى ام ا ، انترليوكين-١ ، تى جى اف ، اف جى اف ، اى جى اف ، و اى جى اف ، والبروتينات هى فيبرونكتين ، فيترونكتين ، كولاجين ، لامينين ، اكتين ، فيتال بروتجلايكان ، وبروتوجلايكان .

بينت الدراسة بان المواد المنشطة للنمو غيرت من معدت انتاج وافراز البروتينات وقد تؤثر هذه على احداث امراض الكلية وتطورها .

ABSTRACT

Mesangial cells, by their unique glomerular location, contractile capacity, cytokine production, adhesion and antigen-presenting capability, are in a pivotal position to initiate and direct renal injury. This study investigate the effect of cytokines on the extracellular matrix proteins produced by stimulated and non-stimulated mesangial cells. The cytokines studied were PMA, IL-1, Il-6, TGF, FGF, IGF, and EGF and the extrcellular matrix proteins fibronectin, vitronectin, collagen IV, laminin, actin, fetal proteoglycan and proteoglycan. APAAP and matrix dot-blot assay were applied. It was shown that cytokines altered the production and secretion of extracellular matrix proteins, these in turn could lead to the alteration of pore size, matrix properties and filtration, and thus influence the development and progression of glmerular diseases.

INTRODUCTION

Mesangial cells, by their unique glomerular location, contractile capacity, cytokine production, adherence, and antigen-presenting capability, are in a pivotal position to initiate and direct renal injury (Brennan et al, 1990).

Cultured human mesangial cells produce a variety of extracellular matrix (ECM) proteins and secretion of some are increased by cytokines (Ischimura et al, 1989).

The aim of this study is to investigate the effect of cytokines on the extracellular matrix proteins produced by stimulated and non-stimulated mesangial cells. The cytokines studied were PMA, IL-1, Il-6, TGF, FGF, IGF, and EGF and the extracellular matrix proteins fibronectin, vitronectin, collagen IV, laminin, actin, fetal proteoglycan, and proteoglycan.

MATERIALS AND METHODS

Cells were harvested and cultivated in RPMI medium (Mukhtar A, 1992 & 1994)

ALKALINE PHOSPHATASE ANTI-ALKALINE PHOSPHATASE IMMUNOSTAINGING (APAAP) USING MULTIPLE CHAMBER SLIDES

Mesangial cells were passaged to Lux multiple chamber slides and grown until they were confluent. The culture medium was poured off and cells washed with PBS. To fix the cells 0.2ml 0.025% glutaraldehyde was added to each well for 20 minutes at room temperature, and the wells were then washed 3 times with TBS. Monoclonal antibodies diluted in TBS (anti-cellular fibronectin (Serotec), anti-collagen IV (Serotec), anti-laminin (Boehringer), anti-vitronectin (Boehringer), anti-proteoglycan (Serotec) and anti-fetal proteoglycan (Serotec) were addded to each well (OX-8, TBS, and RF10 were used as negative controls), and incubated for 1 hour at room temperature. The slides were then washed 3 times with TBS. Rabbit anti-mouse Ig (100μl) was added at room temperature, prior to three washes with TBS. After dilution 1/40 in TBS 100μl of mouse APAAP complexes (Dako) was then added and incubated for 30 minutes at room temperature. The slides again washed 3 times with TBS prior to the addition of 200μl of freshly prepared substrate, veronal acetate buffer with naphthol AS-MX phosphate, levamisole and fast red salt, to each well and incubation for 15-20 minutes at room temerature. The slides were washed with distilled water 2-3 times to stop the reaction then counterstained with haematoxylin, and rinsed with Sco'ts tap water to intensify the blue colour of

the nuclei. Slides were viewed under the light microscope using aqueous mountant.

MATRIX DOT ASSAY

The matrix stored isolated from mesangial cells and stored according to the protocol described by Mukhtar A (1992).
The matrix stored in acetone at -20°C was centrifuged at 3000g for 15 minutes and the supernatant discarded and the the amount 0.1% SDS required to achieve 50-200 G protein/ml was added.
Nitrocellulose sheet was cut to the required size and washed with distilled water and laid on the BioRad dot-blot apparatus. 10-20μl matrix solution was added to each well and incubated for 30 minutes at room temperature. The nitrocellulose sheet was removed from the dot-blot apparatus, blocked with 2% bovine serum albumin (BSA) in Tris-buffered saline (TBS) for 1-2 hours at room temperature and then laid back on the dot-blot apparatus. Hybridoma supernatants (10μl) were added and left at room temerature for 50-60 minutes. Each well then washed 3 times with TBS was added and incubated at room temperature for 30 minutes. After 3 futhur washes with TBS, 10μl of mouse APAAP complexes (Dakopatts), diluted 1:40 in 2% BSA/TBS, was added to each well and incubated for 30 minutes at room temperature. The sheet was then washed 3 times with TBS and once with distilled water. The enzyme substrate was freshly prepared and comprised:
50ml Veronal acetate buffer
25mg Naphthol-as-mx (Sigma)
12 Levamisole (Sigma)

25 mg Fast red salt (Sigma)

The substrate was filtered onto the nitrocellulose sheet, incubated for 3-7 minutes at room temperature, washed with distilled water 4 times and once with tap water. The negative controls were Tgw, RF10 and OX-8 (an irrelavant mouse monoclonal antibody). The dots were scored according to the intensity by two independent observers who were unaware of the distribution test and controls.

GROWTH FACTORS USED

rIL-1 (Boehringer), IL-6 (British Biotechnology), PMA (Boehringer), TGF (R & D system), FGF, IGF and EGF (Boehringer).

RESULTS

1.1 EFFECT OF CYTOKINES ON MESANGIAL CELL CULTURE PROTEINS (1 HOUR GROWTH)

1.1.1 APAAP OF MESANGIAL CELLS GROWN ON CHAMBER SLIDEs

Cellular fibronectin, actin, proteoglycans and laminin were the first proteins detected in the mesangial cells. Cytokines stimulation decreased the intensity of staining of laminin (-) and actin (-) in the the matrix but increased the staining intensity of vitronectin in the matrix and cells (+1).

1.1.2 DOT-BLOT ASSAY OF THE MATRIX

Cellular fibronectin was present in the matrix of non-stimulated and stimulated cells. Actin and laminin were detected in the matrix of non-stimulated cells (+1), while IL-6 increased in the intensity of staining of cellular fibronectin (+2).

1.2 EFFECT OF CYTOKINES ON MESANGIAL CELL CULTURE PROTEINS (3 HOURS GROWTH)

1.2.1 APAAP OF CULTURED MESANGIAL CELLS ON CHAMBER SLIDES

After 3 hours growth all matrix proteins and actin were detectable. Decreased intensity of staining of actin in the matrix of mesangial cells was noted, while fetal proteoglycan was not detectable in the matrix. Cytokines increased the intensity of staining of fibronectin and decreased the collagen IV in the matrix (-). Cytokines stimulation increased vitronectin and decreased actin in the matrix.

1.2.2 DOT-BLOT ASSAY WITH MATRIX HARVESTED FROM CULTURED MESANGIAL CELLS

All matrix proteins except fetal proteoglycans were detected in the matrix of non-stimulated cells. Cytokines stimulation increased the intensity of staining of vitronectin (+3) and proteoglycans but decreased that of collagen IV (-).

1.3 EFFECT OF CYTOKINES ON MESANGIAL CELL CULTURE PROTEINS (6 HOURS GROWTH)

1.3.1 APAAP OF CULTURED CELLS ON CHAMBER SLIDES

All matrix proteins and actin were detectable in the matrix and cells, with staining of collagen IV, proteoglycans (+2), fibronectin (+2) and laminin (+2) more pronounced in the matrix. Cytokine stimulation increased the intensity of staining of vitronectin (+2) in the matrix but decreased that of actin (-).

1.3.2 DOT-BLOT ASSAY WITH MATRIX HARVESTED FROM MESANGIAL CELLS GROWN IN THE FLASKS

All matrix proteins were detected in the matrix of non-stimulated cells. cytokine stimulation increased the intensity of staining of vitronectin (+3) whereas IL-1, IL-6, PMA and TGF decreased the intensity of staining of cellular fibronectin in the matrix (-).

1.4 EFFECT OF CYTOKINES ON MESANGIAL MATRIX PROTEINS AND ACTIN (48 HOURS GROWTH)

1.4.1 APAAP WITH CULTURED MESANGIAL CELLS ON CHAMBER SLIDES

The effect of cytokine stimulahtion was to increase the intensity of staining of cellular fibronectin, fetal proteoglycans (+2), actin (+2) and vitronectin (+2) in both the matrix and cells. Cytokine stimulation increased or did not alter the intensity of staining of proteoglycans in cells and matrix whereas the intensity of staining of laminin and collagen IV did not alter.

1.4.2 MATRIX DOT-BLOT ASSAY

The effect of cytokine stimulation was to increase the intensity of staining of cellular fibronectin (+1) and fetal proteoglycan (+1) in the matrix of mesangial cells whereas such stimulation did not alter the amount of vitronectin, proteoglycan, actin, collagen IV and laminin in the matrix of all cells.

DISCUSSION

The results demonstrated that within the first hour of cellular stimulation in culture, only four of the matrix proteins investigated are produced and secreted in the cells and the matrix of non-stimulated cells (cellular fibronectin, proteoglycan, laminin, and actin). Others are produced later (vitronectin, fetal proteoglycan, and collagen IV). Fetal proteoglycan is produced within 3 hours and detected within cells and matrix. Stimulation of cells with certain cytokines have differential effects on the cells. When culturing non-stimulated cells all the proteins investigated were detected in the matrix and cells within 6 hours of growth. In addition an increase in the intensity of staining of collagen IV, proteoglycan and laminin was detectable in the matrix, and collagen IV was increased within cells. By 48 hours of growth all proteins increased in concentration, except cellular fibronectin which decreased (not detectable) in the matrix of non-stimulated cells.

The concentration of vitronectin in the matrix and cells was not altered within the first 1 hour of growth after stimulation with cytokines, but within 3 hours growth a dramatic increase in the matrix was found. By 48 hours an increase in the vitronectin was observed within the stimulated cells.

Fetal proteoglycan were not initially detected in stimulated mesangial cells and the matrix of non-stimulated cells but were detected by 3 hours inthe stimulated mesangial cells, and after 6 hours in the matrix of stimulated cells.

Cytokines had little effect on actin in cells within 1 hour of growth, but after 3 hours an increase of actin in the matrix of mesangial cells was noted, whereas within 6 hours actin was decreased in the matrix of mesangial cells stimulated with IL-1, PMA and IGF.

Cytokines had little effect on collagen IV in the matrix and cells. After 3 hours there was an increase in the matrix of stimulated mesangial cells, with the highest rise after 48 hours. This increase was noted also in non-stimulated cells.

Proteoglycans were detectable in all stimulated mesangial matrix and cells within 3 hours, and over cells. Laminin was increased in the matrix of stimulated mesangial cells within 3 hours growth compared to 1 hour growth, then increased furthur over 48 hours. As the same number of original cells were studied in all cases, the alterations were thought to be due to production and secretion of proteins. Cytokines, however, do have an effect on the pattern of production and secretion of these cell proteins derived.

Cultured human mesangial cells produce a variety of extracellular matrix (ECM) proteins and secretion of some are increased by cytokines. Ischimura et al (1989) mentioned that collagen IV or laminin secreted by mesangial cells in the substratum accelerates production and secretion of extracellular matrix constituents in an autocrine fashion, and showed that extracellular deposition of collagen IV appeared followed by fibronectin and collagen III. Release of TGF during glomerular injury can mediate accumulation of proteoglycans in the extracellular matrix and predispose the kidney to glomerulonephritis Border et al, 1990). Deregulation production

REFERENCES

-Border W A, Okuda S, Languino L R and Ruolahti E. Transforming Growth factor-B regulates production of proteoglycans by mesangial cells. Kidney Internat. 1990; 37: 689-697.

-Brennan D C, Jeunikar A M, Tahkei F and Reubin-Kelly V E. Mesangial cell acessory functions: Mediation by intercellular adhession molecules -1. Kidney Internat. 1990: 38: 1039-1046.

-Horri Y, Atushi M, Iwano M, et al. Involvement of IL-6 in mesangial proliferative glomerulonephritis. J Immunol. 1989: 12: 3949-3955.

-Ischimura E, Sterzel B, Budde K and Kashgarian. Formation of extracellular matrix by cultured mesangial cells. Am J Pathol. 1989; 134: 843-855.

-Mukhtar A. 1992. Study of glomerulonephritis using monoclonal antibodies against mesangial matrix. PhD thesis. University of Aberdeen. UK.

REFERENCES

-Border W A, Okuda S, Languino L R and Ruolahti E. Transforming Growth factor-B regulates production of proteoglycans by mesangial cells. Kidney Internat. 1990; 37: 689-697.

-Brennan D C, Jeunikar A M, Tahkei F and Reubin-Kelly V E. Mesangial cell acessory functions: Mediation by intercellular adhession molecules -1. Kidney Internat. 1990: 38: 1039-1046.

-Horri Y, Atushi M, Iwano M, et al. Involvement of IL-6 in mesangial proliferative glomerulonephritis. J Immunol. 1989: 12: 3949-3955.

-Ischimura E, Sterzel B, Budde K and Kashgarian. Formation of extracellular matrix by cultured mesangial cells. Am J Pathol. 1989; 134: 843-855.

-Mukhtar A. 1992. Study of glomerulonephritis using monoclonal antibodies against mesangial matrix. PhD thesis. University of Aberdeen. UK.

EFFECT OF CYTOKINES ON MATRIX PROTEINS

DR AHMED MUKHTAR

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تاثير المواد المنشطة على بروتينات الخلايا

المواد المنشطة للنمو لها تاثيرات مختلفة على بروتينات الخلايا ، ولقد تم دراسة هذه التاثيرات على بروتينات وسادة الخلايا . المواد النشطة هى: بى ام ا وانترليوكين ، والبروتينات هى: فيبرونكتين ، كولاجين ، فيترونكتين ، لامينين ، اكتين ، برتوجلايكانز و فى تال بروتوجلايكانز ، والخلايا هى: ميزانجيال ، بى و ، هب جى ٢ ، وبى اج ك .

لقد تبين بان انترليوكين و بى ام ا تزيدان من معدل كولاجين ، بروتوجلايكانز وفيبرونكتين، ولكن لم تاثرا على فيترونكتين ، بينما انترليوكين وبى ام ا قللتا او لم تاثرا على لامينين ، لكن بى ام ا اثرت على زيادة بروتوجلايكانز فى خلايا الميزانجيال .

هذه الدراسة توضح بان المواد المنشطة تاثر على تغير بروتينات وسادة الخلايا وافرازاته مما يؤدى الى تغيرات فى مساحة الفتحات ، خصائص وسادة الخلايا والترشيح ، وهذا بدوره قد ياثر على طبيعة المرض فى الخلايا .

ABSTRACT

Cytokines have different effect on the extracellular matrix proteins. The effect of cytokines on the extracellular matrix proteins produced by a number of diffrerent cell lines was studied. The cytokines were PMA and IL-1, the extracellular matrix proteins fibronectin, collagen IV, vitrinectin, laminin, proteoglcan, actin and fetal proteoglycan. and the cells human mesangial, BeWo, Hep G2 and BHK. APAAP and dot-blot assay were used. It was shown that Il-1 and PMA increased the staining intensity of proteoglacan, fibronectin and collagen IV and did not alter the staining intensity of vitronectin. While PMA and IL-1 decreased or did not alter laminin. PMA increased the staining intensity of proteoglycan in mesangial cells but not altered in cells stimulated with IL-1. This study have shown that cytokines altered the production and secretion of extracellular matrix proteins, these in turn could lead to the alteration of pore size, matrix properties and filtration, and thus influece the pathological conditions of diseases.

INTRODUCTION

Cytokines do have an effect on the pattern of production and secretion of matrix proteins. Muhktar (1992) showed that cytokines alters the extrcellular matrix proteins produced by mesangial cells. The aim of this study is to investigate the effect of cytokines on the extracellular matrix proteins produces by a number of different cell lines. The cytokines were PMA and IL-1, the extracellular matrix proteins fibronectin, collagen IV, vitronectin, laminin, proteoglycan, actin, and fetal proteoglycan and the cells human mesangial, BeWo, Hep G2 and BHK. APAAP and matrix dot-blot assay were used.

MATERIALS AND METHODS

Cells were harvested and cultivated in RPMI 1640 (Mukhtar A, 1992 & 1994).

ALKALINE PHOSPHATASE ANTI-ALKALINE PHOSPHATASE IMMUNOSTAINING (APAAP) USING MULTIPLE CHAMBER SLIDES

Mesangial cells were passaged to Lux multiple chamber slides and grown until they were confluent. The culture medium was poured off and cells washed with PBS. To fix the cells 0.2ml 0.025% glutaraldehyde was added to each well for 20 minutes at room temperature, and the wells were then washed 3 times with TBS. Monoclonal antibodies diluted in TBS (anti-cellular fibronectin (Serotec), anti-collagen IV (Serotec), anti-laminin (Boehringer), anti-vitronectin (Boehringer), anti-proteoglycan (Serotec) and anti-fetal proteoglycan (Serotec) were addded to each well (OX-8, TBS, and RF10 were used as negative controls), and incubated for 1 hour at room temperature. The slides were then washed 3 times with TBS. Rabbit anti-mouse Ig (100μl) was added at room temperature, prior to three washes with TBS. After dilution 1/40 in TBS 100μl of mouse APAAP complexes (Dako) was then added and incubated for 30 minutes at room temperature. The slides again washed 3 times with TBS prior to the addition of 200μl of freshly prepared substrate, veronal acetate buffer with naphthol AS-MX phosphate, levamisole and fast red salt, to each well and incubation for 15-20 minutes at room temerature. The slides were washed with distilled water 2-3 times to stop the reaction then counterstained with haematoxylin, and rinsed with Sco'ts tap water to intensify the blue colour of

the nuclei. Slides were viewed under the light microscope using aqueous mountant.

MATRIX DOT ASSAY

The matrix stored isolated from mesangial cells and stored according to the protocol described by Mukhtar A (1992).
The matrix stored in acetone at -20°C was centrifuged at 3000g for 15 minutes and the supernatant discarded and the the amount 0.1% SDS required to achieve 50-200μG protein/ml was added.
Nitrocellulose sheet was cut to the required size and washed with distilled water and laid on the BioRad dot-blot apparatus. 10-20μl matrix solution was added to each well and incubated for 30 minutes at room temperature. The nitrocellulose sheet was removed from the dot-blot apparatus, blocked with 2% bovine serum albumin (BSA) in Tris-buffered saline (TBS) for 1-2 hours at room temperature and then laid back on the dot-blot apparatus. Hybridoma supernatants (10μl) were added and left at room temerature for 50-60 minutes. Each well then washed 3 times with TBS was added and incubated at room temperature for 30 minutes. After 3 futhur washes with TBS, 10μl of mouse APAAP complexes (Dakopatts), diluted 1:40 in 2% BSA/TBS, was added to each well and incubated for 30 minutes at room temperature. The sheet was then washed 3 times with TBS and once with distilled water. The enzyme substrate was freshly prepared and comprised:

50ml Veronal acetate buffer

25mg Naphthol-as-mx (Sigma)

12mg Levamisole (Sigma)

25mg Fast red salt (Sigma)

The substrate was filtered onto the nitrocellulose sheet, incubated for 3-7 minutes at room temperature, washed with distilled water 4 times and once with tap water. The negative controls were TBS, RF10 and OX-8 (an irrelavant mouse monoclonal antibody). The dots were scored according to the intensity by two independent observers who were unaware of the distribution test and controls.

GROWTH FACTORS USED

rIL-1 (Boehringer) and PMA (Boehringer).

CELL LINES

Mes (Human glomerular mesangial cells), BeWo (Choriocarcinoma, Human, Cancer Research. 28: 1231: 1986), Hep G2 ((Human hepatocellular carcinoma: Nature. 282: 616, 1979), and BHK (Fibroblast, baby hamster kidney).

RESULTS

EFFECT OF CYTOKINES ON MATRIX PROTEINS

APAAP ASSAY USING CELL CULTIVATED ON CHAMBER SLIDES (48 HOURS GROWTH)

In an APAAP assay using an anti-fibronectin antibody the different effects of PMA and IL-1 on each cell were demonstrated (Table 1.1.1-4). IL-1 increased staining for fibronectin in cells and matrix. Both PMA and IL-1 decreased expression of proteoglycans and collagen IV in the cells and the matrix but increased or did not alter staining for vitronectin in the matrix. Expression of the later, however, was decreased in the cells. The effect of PMA and IL-1 on fetal proteoglycan expression was different for each cell line: in mesangial cells staining for fetal proteoglycan was increased in the cells stimulated with PMA (+3) but was not altered in the cells stimulated with IL-1 (+3). Laminin was decreased within mesangial cells stimulated with IL-1 (+2) but was not altered in cells stimulated with PMA (+3). In the matrix, however, of cells stimulated with PMA it was increased (+1) but was not altered in the matrix of cells stimulated with IL-1.

DOT-BLOT ASSAY WITH MATRIX PROTEINS AND ACTIN HARVESTED FROM TISSUE CULTURED FKASKS (48 HOURS GROWTH)

Using a dot-blot assay the effect of PMA and IL-1 on cellular protein synthesis was again estimated. Fibronectin was not altered (+3) in the matrix of mesangial cells by either cell stimulator whereas both increased proteoglycan expression in the cell matrix.

This contrast with the decrease in collagen expression in the matrix of various cell types after stimulation with both PMA and laminin in the matrix of cell lines (+1).

DICUSSION

The study showed that cytokines (PMA and IL-1) have different effect on extracellular matrix proteins of different adherent cell lines. After 48 hours cultivation, it was shown that IL-1 and PMA increased the staining intensity of fibrnectin, proteoglycan and collagen IV in the cells and matrix but did not alter the staining of vitronectin in the matrix. While PMA and IL-1 have different effect on fetal proteoglycan expression: in mesangial cells it was increased in the cells stimulated by PMA and was not altered in the cells stimulated with IL-1. PMA and IL-1 decreased or did not alter the staining intensity of laminin.

It was shown that IL-1 posses activity in a thymocyte-co-mitogen assay, to be capable of stimulating the release of IL-1 from splenic T cells, and to have intrinsic activity as an endogeneous pyrogen (Lovett et al, 1983). Deregulation production of IL-6 may be involved in proliferative glomerulonephritis (Horri et al, 1989).

This study showed that cytokines altered the production and secretion of extracellular matrix proteins, these could in turn could lead to the alteration of pore size matrix, properties and filtration and thus influence the pathological conditions of certain diseases.

REFERENCES

-Horri Y, Atushi M, Iwano M, et al. Involvement of IL-6 in mesangial proliferative glomerulonephritis. J Immunol. 1989: 12: 3949-3955.

-Mukhtar A. Study of glomerulonephritis using monoclonal antibodies against mesangial matrix. PhD thesis. University of Aberdeen: 1992. UK.

-Mukhtar A. 1994. Expression of MP84 in the biopsies section of benign and malignant tumours, Accepted for publication in Hamdard Medicus: 1994.

-Lovett D H, Ryan J L, Dterzel R B and Attkins E. Stimulation of rat mesangial cell proliferation by macrophage interleukin-1. J Immunol. 1983; 131: 2830.

Table 1.1.1 EFFECT OF CYTOKINES ON ADHERENT CELLS IN THE CULTURE (APAAP)

CELL	Fibronectin			Actin		
	NS	PMA	IL-1	NS	PMA	IL-1
G402	(+1)	(+3)	(+2)	(+1)	(+2)	(+1)
	[+1]	[+1]	[+2]	-	-	[+1]
Hep G2	(+3)	(+2)	(+2)	(+1)	(+1)	(+1)
	[+3]	[+1]	[+3]	[+1]	[+1]	[+1]
HFL-1	(+3)	(+2)	(+3)	(+3)	(+2)	(+1)
	[+3]	[+1]	[+3]	[+1]	-	[+1]
Hu Fib	(+2)	(+2)	(+2)	(+1)	(+1)	(+1)
	[+1]	[+1]	[+1]	-	-	-
Mes	(+3)	(+3)	(+3)	(+4)	(+2)	(+3)
	[+2]	[+3]	[+3]	[+1]	[+2]	[+3]
BeWo	(+2)	(+2)	(+3)	(+1)	(+3)	(+2)
	[+3]	[+3]	[+3]	[+1]	[+1]	[+1]
293	(+1)	(+2)	(+3)	(+2)	(+1)	(+3)
	[+3]	[+1]	[+3]	[+1]	-	[+1]

NS=non-stimulated, PMA='stimulated with $10x^{-5}$M PMA, IL-1= stimulated with 10U/ml of rIL-1ß. ()=cells, []=matrix.

Table 1.1.4 EFFECT OF CYTOKINES ON ADHERENT CELLS IN CULTURE (APAAP)

CELL	Laminin			OX-8		
	NS	PMA	IL-1	NS	PMA	IL-1
G402	(1)	(3)	(1)	-	-	-
	-	-	-	-	-	-
Hep G2	(1)	-	(3)	-	-	-
	-	-	-	-	-	-
HFL-1	(1)	(1)	(1)	-	-	-
	-	-	-	-	-	-
Hu Fib	(1)	-	-	-	-	-
	-	-	-	-	-	-
Mes	(3)	(3)	(2)	-	-	-
	-	[1]	-	-	-	-
BeWo	(2)	(1)	(1)	-	-	-
	-	-	-	-	-	-
293	-	(1)	(2)	-	-	-
	-	-	-	-	-	-

NS=non-stimulated, PMA=stimulated with $10x^{-5}$M PMA, IL-1= stimulated with 10U/ml of rIL-1ß. ()=cells, []=matrix.

Table 1.1.3 EFFECT OF CYTOKINES ON ADHERENT CELLS IN CULTURE (APAAP)

CELL	Vitronectin			Fetal Proteoglycan		
	NS	PMA	IL-1	NS	PMA	IL-1
G402	(1)	(1)	-	(2)	(3)	-
	-	-	-	[1]	[1]	-
Hep G2	(1)	-	-	(1)	(1)	(1)
	-	-	-	[1]	[1]	[1]
HFL-1	(1)	(1)	(1)	(2)	(1)	(1)
	-	-	-	-	-	[2]
Hu Fib	-	(1)	-	(2)	(2)	(1)
	-	-	-	[1]	[1]	[1]
Mes	(2)	(1)	(1)	(2)	(3)	(2)
	[1]	-	[1]	[1]	[3]	[1]
BeWo	(1)	-	(1)	(1)	(1)	(3)
	-	[2]	[2]	[3]	[2]	[2]
293	-	(2)	(1)	(1)	(1)	(1)
	-	-	-	[1]	[3]	[2]

NS=non-stimulated, PMA= stimulated with $10x^{-5}$M PMA, IL-1= stimulated with 10U/ml of rIL-1ß. ()=cells, []=matrix.

Table 1.1.2 EFFECT OF CYTOKINES ON ADHERENT CELLS IN THE CULTURE (APAAP)

CELL	Proteoglycans			Collagen IV		
	NS	PMA	IL-1	NS	PMA	IL-1
G402	(2)	(3)	(1)	(1)	(1)	(2)
	[1]	[1]	-	-	[1]	-
Hep G2	(2)	(1)	(1)	(1)	-	(1)
	[1]	-	[1]	[1]	-	-
HFL-1	(3)	(3)	(1)	(3)	(2)	(2)
	-	-	-	-	-	-
Hu Fib	-	(1)	-	(1)	(1)	-
	-	-	-	-	[1]	-
Mes	(3)	(3)	(3)	(2)	(2)	(1)
	[3]	[2]	[1]	[1]	[1]	-
BeWo	(2)	(2)	(3)	(1)	(3)	(2)
	[1]	[2]	[1]	-	[1]	-
293	(3)	(3)	(1)	(1)	-	(1)
	[1]	[1]	[1]	[1]	-	[1]

NS=non-stimulated, PMA= stimulated with $10x^{-5}$M PMA, IL-1= stimulated with 10U/ml of rIL-1ß. ()=cells, []=matrix.

RENAL TRANSPLANTATION

دراسات طبية عن زراعة الكلية

هذه االدراسات تبين:

١. ان بول الاشخاص الذين تم زراعة كلى لهم والمصابين بالرفض الحاد يحتوى على مادة انترليوكين ١.

٢. ان انسجة الاشخاص الذين تم زراعة كلى لهم والمصابين بالتهاب المناعى أ للكلية تكون مادة بروتيوجلايكان.

٣. ان انسجة الاشخاص الذين تم زراعة كلى لهم والمصابين بالرفض المزمن تحتوى على مادة فيبرونيكتين.

٤. ان كريات الدم البيضاء للاشخاص الذين تم زراعة كلى لهم تحتوى على مادة ام بى ٤٨.

INTERLEUKIN-1 EXCRETION IN URINE SPECIMENS OF RENAL TRANSPLANT RECIPIENTS WITH ACUTE REJECTION USING MATRIX DOT BLOT ASSAY TEST

DR AHMED MUKHTAR

Interleukin-1 is a polypeptide mediator centrally involved in the regulation of inflammatory response. kidney mesangial cells produces IL-1 (lovett et al, 1988). This study was performed to determine the excretion of IL-1 in the urine of renal transplant recipients with acute rejection. Six persons with renal transplant acute rejection and 10 healthy persons were included. Two urine specimens from each person were collected. matrix dot-blot assay was performed. it was shown that 12 urine specimens from 6 persons with acute rejection revealed IL-1 in the matrix dot-blot assay while there was no staining for IL-1 in the urine specimens of healthy persons. This could be due the immunological alteration during the acute rejection which could lead to autocrine and paracrine secretion of IL-1 in the urine. The mechanism of IL-1 secretion is not clear.

Reference:

Lovett DH, Martin M, Bursten S, et al. Interleukin 1 and the glomerular mesangium: III. IL-1 dependent stimulation of mesangial cell proliferation kinase activity. Kidney Internat. 1988: 34: 26-35.

HEPARAN SULPHATE PROTEOGLYCANS EXPRESSION IN IGA NEPHROPATHY KIDNEY SECTION FROM A PATIENT WITH 4 YEARS SUCCESSFULL RENAL TRANSPLANTATION.

DR AHMED MUKHTAR

Proteoglcans are glycoprotens to which polyanionic glomerular chondrotin sulphate proteoglycans are synthesized. Heparan sulphate proteoglycans (HSPG) is present in all normal and nephrotic glomeuli, it is found predominantly in the epithelial aspect of the glomerular basement membrane and mesangium (Moss et al, 1990). The aim of this study is to detemine the distribution of HSPG in IgA nephropathy kidney sections from a patient with IGA nephrapathy, his previous nephrectomy was due to end-stage IgA nephropathy. Immunofluorescent antibody test was used. it was shown that HSPG distribution is different from previous studies with strong localization in glmerular basement membrane and mesangium.

References:

Moss J, Woodrow DF, Shore I, Phillips M and Spiro RG. Ultrastructure immunogold studies of heparan sulphate proteoglycan in noormal human glomeruli and glomerulonephritis. J pathol. 1990; 161: 137-143.

VITRONECTIN LOCALIZATION IN BIOPSY SECTIONS OF CHRONIC REJECTED TRANSPLANT RECIPIENT.

DR AHMED MULHTAR

Immunohistochmeical studies of S protein (vitronectin) in normal and diseased human kidneys showed that S protein was diffusly present in arteriolar perimocytic matrices and in the cortex of normal and diseased kidneys. It is an adhesine glycoprotein and it was observed a strong co-staining of immune deposits for S protein and C5b-9 in normal and diseased kidney sectios (Lelongt et al,1987). This is the first study of vitronectin protein in kidney sections of chronic rejected kidney transplant recipient. Immunofluorescent antibody test was used. It was shown that kidney sectios were strongly stained in the mesangium. This distribution and co-existance of S proten with C5b-9 could represent membrane cytolytic complement complexes.

References:

Lelongt B, Makino H and Kanwar YS. Status of glomerular proteoglycans in aminonucleoside nephrosis. Kidney Internat. 1987; 31: 1299-1310.

MP48 EXPRESSION IN RED BLOOD CELLS OF RENAL TRANSPLANT RECIPIENTS

DR AHMED MUKHTAR

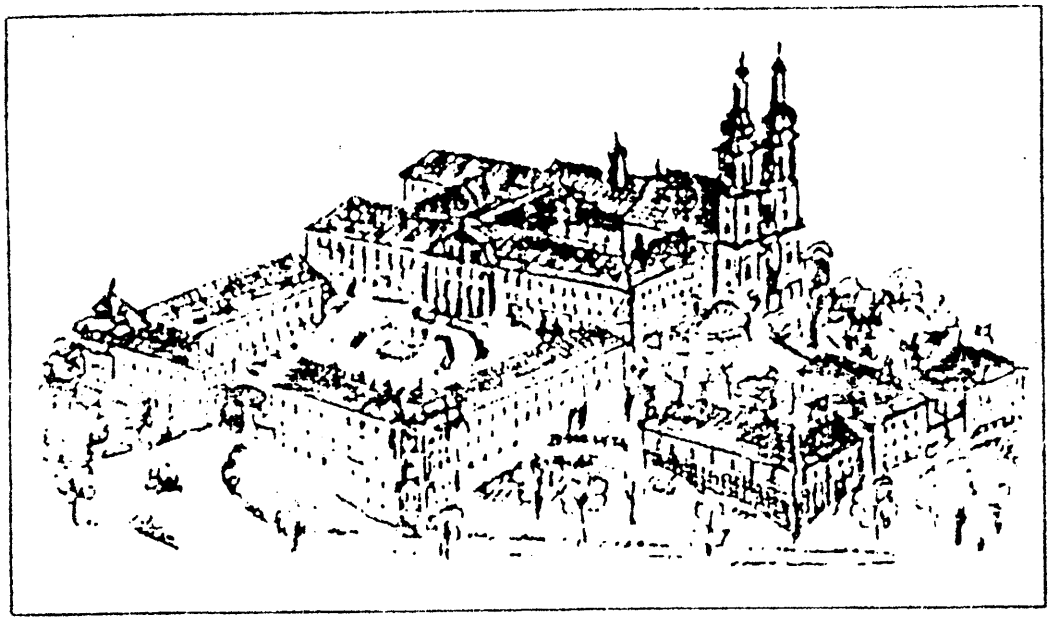
MP48 is a protien expressed in diseased kidney sections but not in the normal kidney sections. It is also exoressed in the spleen, liver and pancreas but not in the erythrocytes of healthy persons (Mukhtar A, 1992). This study shows that MP48 is expressed in the erythrocytes of renal transplant recipients using an EPICS-C flow cytometer (Coulter Immunology). The expression of this antigen amongst renal transplant recipients reveals some pathological and immunological alteration amongst renal transplant recipients for the production of MP48 protein.

Reference:

Mukhtar A. Study of glmerulonephrits using monoclonal antobodies against mesangial matrix. PhD Thesis. University of Aberdeen, 1992.

MESANGIAL CELLS AND EXTRACELLULAR MATRIX

a Forefronts in Nephrology Symposium of the International Society of Nephrology

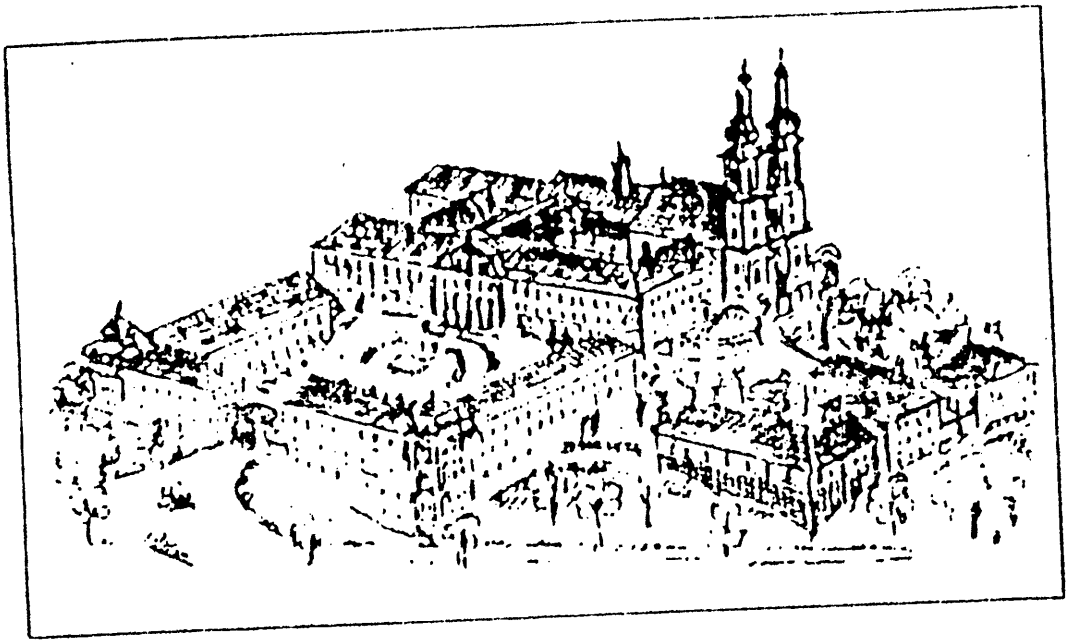


June 9 - 12, 1991
Kloster Banz

near Erlangen-Nürnberg, Germany

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PRODUCTION OF MONOCLONAL ANTIBODIES TO STIMULATED MESANGIAL CELL MATRIX

DA Power, A Mukhtar, MC Jones. Department of Medicine and Therapeutics, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen AB9 22 D.

The mesangial cell is thougt to play a pivotal role in many forms of glomerulonephritis. We have produced a series of monoclonal antibodies to the extracellular matrix (ECM) deposited by mesangial cells which have been stimulated with phorbol ester or IL-1. Six of these react with ECM from stimulated but not normal cultured mesangial cells. The antigen detected by one of these MAb is upregulated by all six cytokines tested (EGF, IL-1, IL-6, TGFB, IGF-1, PDGF and FGF) and its expression can be prevented by inhibitors of gene transcription (actinomycin D) and translation (cycloheximide and puromycin). Apart from the choriocarcinoma cell line BeWo, it is unique to cultured mesangial cells. The antibody does not stain normal kidney sections but renal biopsies showing IgA nephropathy and membranous glomerulonephritis are strongly stained within the glomerulus. On reduced Western blots it detects three clear bands of 150, 115 and 95 kD. The identity of this antigen is unknown but it may be the first example of a molecule whose expression is limited to stimulated mesangial cells and diseased glomeruli.

SCOTTISH SOCIETY FOR EXPERIMENTAL MEDICI

A scientific meeting will be held in Aberdeen (Medi Chirurgical Hall, Aberdeen Royal Infirmary, Foresterh on Friday 1st March 1991 starting at 10.30 a.m. Ple: complete and return the enclosed card to Dr David Reid Tuesday 26th February.

Agenda for Annual General Meeting (1.45 p.m.)

1. Apologies.
2. Minutes of last meeting.
3. Secretary's report.
4. Treasurer's report.
5. Nominations for membership—Drs A. B. Bridge Tessa Holyoake, M. Morrison, Elizabeth Murph A. J. Pelosi, N. Ross Hunter, T. Walsh.
6. Resignations – Dr. G. J. Addis, Mr. H. I. Tankel.
7. Any other business.

This meeting kindly sponsored by **SYNTEX**

Next meeting Glasgow Friday 10th May

P. F. Semple
Hon. Secretar

TRAIN TIMES

From	Dundee	dep.08.46	arr. 10.03
	Edinburgh	dep.07.29	arr. 10.03
	Glasgow	dep.07.15	arr. 10.03

with methacholine and dose-response curves constructed to isoprenaline (ISO), SNP and ANF. Results are expressed as the concentration of each relaxant producing a 30% relaxation of induced tone (IC_{30}), and also as the percentage relaxation of induced tone produced by 10^{-5} M of each agent (n=6).

	IC_{30}	% Relaxation (10^{-5}M)
ISO	7.38 (0.13)	207 (35)
SNP	6.25 (0.36)*	154 (39)
ANF	6.33 (0.29)*	85 (17)*

* p<0.03 vs isoprenaline

Electrical field stimulation (16Hz, 0.1ms, 50V) showed no attenuation of response by 10-6 ANF (n=6).

We conclude that both ANF and SNP have a direct relaxant effect on methacholine induced tone in human bronchial smooth muscle but are less potent than isoprenaline.

INDOMETHACIN AUGMENTS ANGIOTENSIN II INDUCED ANTINATRIURESIS AND RENAL VASOCONSTRICTION IN MAN. J. G. Motwani, A. D. Struthers, *Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee.*

We investigated whether vasodilator prostaglandins are important in counter-balancing the renal vasoconstrictor effects of angiotensin II (AII) in man. Eight volunteers underwent over-hydration on four mornings. The prostaglandin synthetase inhibitor indomethacin 100mg or placebo was given orally and either placebo or AII 1ng per kg per minute infused. Indomethacin and AII toghether reduced sodium excretion and renal perfusion to a much greater extent than AII alone with an additional fall in glomerular filtration rate (GFR) not found with AII alone (all p<0.005). Thus in the presence of high circulating AII levels, as in chronic heart failure, vasodilator prostaglandins may play an important role in maintaining GFR by offsetting afferent renal arteriolar constriction.

EVALUATION OF IN-VIVO PARTIAL AGONIST ACTIVITY: DOSE-RANGING STUDY WITH CARTEOLOL. N. M. Wheeldon, D. G. McDevitt, B. J. Lipworth, *Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee.*

We investigated methods of assessing partial agonist activity (PAA) *in vivo* using increasing doses of carteolol.

Eight normal volunteers took single oral doses of carteolol 10mg (C10), 30mg (C30), 60mg (C60); pindolol 30mg (P30); nadolol 40mg (N40) and placebo (PL).

Carteolol displayed dose-related increases in resting heart rate, systolic blood pressure and attenuation of exercise hyperkalaemia. Carteolol did not produce dose-related reduction in exercise heart rate. Both C60 and P30 significantly (p<0.01) increased finger tremor. All treatments significantly blunted terbutaline-induced hypokalaemic, chronotropic and finger tremor effects (p<0.001). Carteolol is thus a non-selective β-antagonist with both β_1 and β_2-PAA but β_1-PAA predominates at conventional clinical doses. Attenuation of exercise hyperkalaemia is a novel method of evaluating β_2-PAA *in vivo*.

Afternoon Session

CHAIRMAN: Professor R. L. Himsworth

DIPYRIDAMOLE INCREASES RED CELL DEFORMABILITY. A. Sariabadi, A. Bridges, J. Taylor, C. Lau, J. Belch, C. Forbes, *University of Dundee, Department of Medicine, Niewells Hospital, Dundee.*

The average diameter of nutritive capillaries is about half that of red blood cells (RBC), therefore, RBC deformability is essential for optimal microvascular perfusion. We have developed an automated technique for measuring RBC deformability ex-vivo. RBC deformability was studied in patients on haemodialysis (HD) due to chronic renal failure (n=18); patients with peripheral vascular disease (PVD, n=23) and matched controls (n=33, hospital outpatients). RBC deformability in HD and PVD group was significantly lower (p<0.0005) as compared with the control group. When the red cells were treated with 5μM dipyridamole, their deformability increased markedly, p<0.0005, p<0.0001, p<0.0001 for controls, HD and PVD respectively as compared with untreated cells. The results suggest that dipyridamole may be beneficial if more-rigid red cells contribute to impaired microvascular perfusion.

2.15 PRODUCTION OF MONOCLONAL ANTIBODIES TO STIMULATED MESANGIAL CELL MATRIX. M. C. Jones, A. Mukhtar, D. A. Power, *Departments of Medicine and Therapeutics, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen.*

The mesangial cell is thought to play a pivotal role in many forms of glomerulonephritis. We have produced a series of monoclonal antibodies to extracellular matrix (ECM) from cultured mesangial cells stimulated with phorbol ester or IL-1. Six of these react with stimulated but not normal cultured mesangial cells. The antigen detected by one these MAb is upregulated by all six cytokines tested and requires both gene transcription and translation for expression. Macrophages and BeWo cells express this antigen but is not found on other normal or stimulated cell lines. On normal kidney sections the MAb reacts with macrophages alone. In various disease states, however, there is significant reactivity with cells within the glomerulus. This MAb may be useful, therfore, in determining responsiveness of mesangial cells to cytokine stimulation.

2.30 THERE IS A DIURNAL VARIATION IN THE MARKERS OF FREE RADICAL ACTIVITY IN HEALTHY VOLUNTEERS. P. Ventresca, C. Duthie, J. McLay, *Department of Medicine and Therapeutics, Aberdeen Royal Infirmary and Rowett Research Institute, Aberdeen.*

Markers of free radical activity, are commonly measured and related to either a disease process or drug treatment. No study to date has examined the possible existence of a diurnal variation in these parameters or attempted to optimise the conditions under which samples for free radical measurement should be taken. This study measured plasma levels of MDA, conjugated dienes, vitamin E, vitamin C, and cholesterol in 10 healthy volunteers over two separate 24 hour periods. Markers of free radical activity MDA and conjugated dienes, and vit C exhibited a significant diurnal variation with minimum values in the morning (8am) and peak values in the early evening (7pm). Compared to baseline (8am), peak plasma levels (7pm) of MDA., conjugated dienes, and vitamin C increased by 25.7 % , 53.1% and 13.9% respectively (p<0.01). Markers of free radical activity were reproducible only if measured at 8am after an overnight fast.

2.45 SENSITIVITY OF TOMOGRAPHIC RADIONUCLIDE BLOOD POOL IMAGING FOR THE DETECTION OF OCCULT CORONARY ARTERY DISEASE. S. Cross, M. J. Metcalfe, M.Y. Norton, K. Jennings, S. Walton, *Cardiac Department, Aberdeen Royal Infirmary, Aberdeen.*

Tomographic blood pool imaging is a new technique which may be of use for the detection of occult coronary artery disease (CAD). To investigate this, we have studied 100 consecutive patients undergoing routine cardiac catheterisation. 63 had prior MI, 31 had proven CAD and six were normal.

Using the Aberdeen Section Scanner, matching "bullseye" images of abnormalities of left ventricular wall motion were derived by reference to pooled data from 50 control subjects.

For patients with prior MI, angiography detected 97 per cent and tomography 89 per cent, for CAD the figures were 19 per cent and 71 per cent. For the normals no abnormality was detected using either method.

Tomographic imaging is similar to angiography for the detection of prior MI but is superior for occult CAD.

3.00 TIN PROTOPORPHYRIN COMBINED WITH HAEM ARGINATE: AN IMPROVED TREATMENT FOR ACUTE PORPHYRIA. S. B. Dover*, M. R. Moore, Anne Graham, E. L. McColl, *University Department of Medicine and Therapeutics Western Infirmary, Glasgow.*

Haem arginate, as a treatment for acute intermittent porphyria, transiently suppresses the over-production of haem precursors, 5-aminolaevulinate (ALA) and porphobilinogen (PBG)[1]. We investigated the possibility of augmenting this effect with tin-protoporphyrin, an inhibitor of haem-oxygenase. Five patients were treated in separate attacks with placebo, haem arginate alone and then combined with tin-protoporphyrin. On the third day of treatment the mean urinary excretions of ALA and PBG were 86 per cent and 73 per cent of pre-treatment values on placebo, 15 per cent and 24 per cent with haem arginate alone, and 11 per cent and 15 per cent with the combination. Five days later the values were placebo, 90 per cent and 77 per cent, haem arginate 49 per cent and 54 per cent and the combination 14 per cent and 15 per cent. All patients followed this pattern. This extended biochemical response was mirrored in prolonged clinical remission in a patient with frequent attacks of acute intermittent porphyria (AIP).

Reference

1. A. L. Herrick, M. R. Moore, K. E. L. McColl, A. Cook, A. Goldberg. Controlled trial of haem arginate in acute hepatic porphyria. Lancet 1989: 1. 1295-1297.

*Introduced

15 **SYMPTOMS AND PHYSIOLOGICAL RESPONSES TO ACUTE INSULIN-INDUCED HYPOGLYCAEMIA IN NORMAL HUMANS.** K. M. MacLeod, D. A. Hepburn, N. Powers, L. A. Arblaster, E. G. Walshe, B. M. Frier, *Department of Diabetes, Royal Infirmary Edinburgh and Departments of Physiology, University of Edinburgh and University of St. Andrews.*

An acute autonomic reaction (R) occurs when blood glucose declines to a specific concentration. Although R can be identified by the autonomic symptoms of hypoglycaemia, objective physiological changes may identify R with greater precision. Acute hypoglycaemia was induced in eight healthy subjects with i.v. insulin (2.5 mU/kg/min) and heart rate, blood pressure, sweating and tremor were measured along with symptom scores, plasma glucose and catecholamine concentrations. All physiological responses occurred simultaneously, coincidental with the onset of symptoms (blood glucose 1.6±0.2 mmol/l); considerable variation in the intensity of each response was observed between individuals. These objective physiological measurements may be used to identify a putative alteration in the glycaemic threshold for R in diabetic patients with hypoglycaemia unawareness.

10 **Poster Discussions**

5 **Tea**

MP/84 ANTIGEN EXPRESSION IN BIOPSY SECTIONS OF PATIENTS WITH BENIGN AND MALIGNANT TUMOURS

AHMED MUKHTAR AND AHMED AL-ARRAYYED

This study indicates that MP/84 antigen is expressed in the kidney sections of renal cell carcinoma and it is not expressed in any other acute or malignant tissues sections. MP/84 is an antigen expressed in diseased kidney sections such as IgA nephropath, membranous and minimal change. It was noted from previous studies that it is also expressed in BeWo choriocarcinoma cell line and not in other cell tested. The pathogenesis mechanism could be due to the secretion of cytokines by the renal cells.

Introduction

Tumours from defined and those from which etiological hypothesis can be deducted such as environmental origin play an important factor in the growth and development of tumours. The problem of generation of neoplasms from initiated cells is a central one in carcinogenesis. Whether a cell is initiated by germinal mutation or by somatic mutation, that event is insufficient to produce a malignant tumour (Knudson, A.G., 1981).

Growth factors and growth factor receptors are or may be involved in the abnormal growth characteristic of cancer, and both entities are encoded by known proto-oncogens.

MP 84/15 is a protein synthesized in response to several cytokines (EGF, PDGF, IGF-1, IL-1, IL-6, FGF and TGF). It is produced by the mesangial cells within 15 minutes and secreted into the matrix within 30 minutes when the cells are stimulated *in vitro*. This antigen is present only in diseased kidney sections and choriocarcinoma BeWo cell line (Mukhtar, A., 1991 and 1993). Its expression in tumour tissues sections is unknown.

Materials and Methods

Tissue sections

Tissues sections of the following tumours were used in the study,

and FITC was per...
cell carcinoma, Benign prostatic hyperplasia, Malignant lymphoma (Intermediate grade), Conjunctival squamous cell carcinoma, Transitional cell carcinoma, Dermatofibroma, Acute lymphoblastic leukaemia, Basal cell carcinoma (Skin), Squamous carcinoma (Cervix), Transitional cell carcinoma (Urinary bladder), Serous cystadenoma (Ovary), Subependymoma (Cerebellum), Pyogenic granuloma, Non-Hodgkin's lymphoma (Rectum), Squamous cell carcinoma (Uterine cervix), Carcinoma (Pleura), Mucinous carcinoma (Stomach), Malignant vasoformative tumour, Fibroadenoma (Breast), Papillary cell carcinoma Grade II (Bladder), Invasive cell carcinoma (Cervix), Squamous cell carcinoma (Gum), Renal cell carcinoma, adenocarcinoma (Skin), Squamous cell carcinoma grade II (Tongue), Benign juvenile melanoma "Spitz Naevus" (Skin), Renal cell adenoma.

Immunostaining of Tissue sections Fixed with Paraformaldehyde: Frozen sections were fixed for 20 minutes 2% with (wt/vol) paraformaldehyde in PBS, incubated for 20 minutes, quenched with ammonium chloride, and permeabilized for 1 hour with PBS containing 0.01% (wt/vol) saponin and 0.25% (wt/vol) bovine serum gelatinin (PBS/sap/gel). Wells were incubated with monoclonal antibodies supernatants, with ascitic fluid or serum diluted in PBS/sap/gel (TBS, RF10 and OX-8 were used as negative controls) for 1 hour, washed with PBS/sap/gel 100 1 of anti mouse Ig FITC conjugate (TACO, Inc), which has been diluted in 1/80 in Evans blue dye in PBS/sap/gel was applied. After incubation for 30 minutes at room temperature the slides were washed 3x with PBS/sap/gel and sealed under 10% glycerol in PBS containing 1,4 diazabicyclo-(2,2,2) octane (Polyscience, Inc) to minimize quenching, and examined under fluorescent microscore-Olympus, BH2 (Burkhadt, 1989 and Mukhtar, 1992).

Results

There was no staining of any tissue section used in this study except renal cell carcinoma as follows: All tissues of the renal cell carcinoma were not stained except the glomerulus. Glomerular basement membrane (+3), mesangial cells (+2), mesangial matrix (+3), epithelial cells (+2) and endothelial cells (+2). Generally adense granular staining of the glomerular basement membrane and cells was noted.

Discussion

This study showed that MP/84 is expressed in the kidney biopsy sections with renal cell carcinoma. The expression of the antigen was strictly located in the glomerulus, with strong glomerular ...sangial cell and matrix expression. This other malignant tumours sections studied.

Cytokines are produced in the response to the mesangial cellular injury (Sedor, J.R., 1992), and these alter the mesangial and extracellular matrix components (Sterzel, R.B., Schulze-Lohoff, E. and Marx, 1993). The molecular and biochemical events, which mediate cytokine-induced transition are poorly understood. This process involves induction of aspecific gene set by a network of a short-term signals generated by interaction of the cytokine with the binding domain of its receptor.

The expression of MP/84 antigen in the renal cell carcinoma kidney section could be in by same process as in primary glomerulonephritis kidney (Mukhtar, A., 1993). Its relation to its expression in BeWo choriocarcinoma cell line is not understood since no section of choriocarcinoma was used in this study. However, this study shows that MP/84 antigen is only expressed in the diseased kidney section including malignant tumours of the renal such as renal cell carcinoma.

REFERENCES

1. Burkhadt, J.K., Hester, S. and Argon, Y. The glycoprotein of VSV accumulates in a distal golgi compartment in the presence of CCCP, *J. Cell. Sci.*, 1989, 92:643-645.
2. Glynn, A.A., Brumfitt, W. and Howard, C.J. Antigens of Escherichia coli and renal involvement in urinary tract infections. *Lancet.* 1:514-516, 1971.
3. Mukhtar, A. Study of glomerulonephritis using monoclonal antibodies to mesangial matrix, *Ph.D thesis,* University of Aberdeen, 1992.
4. Power, D.A., Mukhtar, A. and Jones, M.C. Production of monoclonal antibodies to stimulated mesangial cell matrix (Abstract). Kidney International, 1992, 41(3):703.
5. Sendor, J.R. Cytokines and growth factors in renal injury. *Semin Nephrol,* 12:428-442, 1992.
6. Sterzel, R.B., Schulze-Lhoff, E. and Marx, M. Cytokines and Mesangial Cells, Kidney Int., 39:26-31, 1993.
7. Tisher, C.C. and Benner, B.M. *Renal Pathology with Clinical Functional Correlation,* Vol. I, J.B. Lippincott Company, 1989, pp. 777-808.

KIDNEY INTERNATIONAL

Volume 41, Number 3, March 1992

Official Journal of the International Society of Nephrology

Le Journal Official de la Société Internationale de Néphrologie

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Kidney International is published monthly by Blackwell Scientific Publications, 3 Cambridge Center, Cambridge, MA 02142, U.S.A. Tel (617) 225-0401. ISSN 0085-2538. U.S.P.S. 091-170.

Printed in the United States. Second-class postage paid at Boston, MA and additional mailing offices.

Change of address: Allow six weeks for all changes to become effective. All communications should include both old and new addresses (with zip codes) and should be accompanied by a mailing label from a recent issue.

Postmaster: Please send changes of address to Kidney International, Blackwell Scientific Publications, 3 Cambridge Center, Cambridge MA 02142, U.S.A.

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Production of monoclonal antibodies to stimulated mesangial cell matrix. *D.A. Power, A. Mukhtar, M.C. Jones, Department of Medicine and Therapeutics, Aberdeen Royal Infirmary, Aberdeen, Scotland, United Kingdom.* The importance of cytokines in the pathogenesis of mesangial proliferative glomerulonephritis is unclear, despite evidence of extensive cytokine responsiveness and autocrine production of cytokines in vitro. To detect the action of cytokines in vivo, we have attempted to produce monoclonal antibodies which react with proteins secreted de novo into the extracelluiar matrix (ECM) by cultured mesangial cells following cytokine stimulation. ECM was obtained from cultured mesangial cells stimulated with phorbol ester or IL-1 and used to immunize mice. Hybridomas were produced and screened by dot blot assay using ECM from stimulated and non-stimulated cells: those hybrids which reacted with ECM from stimulated cells alone were chosen for further study. Six separate antibodies were obtained which reacted with ECM and cultured mesangial cells only after stimulation. The antigen detected by one of these, MP15/84, is synthesized in response to all cytokines tested (EGF, PDGF, IGF-1, IL-1, IL-6, FGF, TGF-β), in response to low concentrations of IL-1 (0.5 to 1.0 μ/ml) and within 15 min. of stimulation. The antigen was not present in cycling cells grown in FCS but required both gene transcription and translation for expression. The choriocarcinoma cell line BeWo expressed it constitutively but a variety of other transformed and normal cell types did not. MP15/84 detected three bands of M_r 161, 128 and 97 kD on Western blots; the M_r was identical on reduced and non-reduced gels.

The antigen was expressed in kidneys with IgA nephropathy and membranous glomerulonephritis but not in normal kidneys or in a number of normal tissues. These studies suggest that novel extracellular matrix proteins are produced in glomerulonephritis, perhaps in response to cytokine stimulation.

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6 June 1991

Dr A Mukhtar
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Aberdeen
AB9 2ZD

Dear Dr Mukhtar

"MECHANISMS OF VASCULAR INJURY IN RENAL DISEASE"
One Day Workshop

Wednesday 6 November 1991
Lecture Theatre
Institute of Urology
172 Shaftesbury Avenue
LONDON WC2

This year's meeting will be held in the Lecture Theatre at The Institute of Urology.

The format will be as before, i.e. 10-minute presentations followed by 10 minutes for discussion. Although the title of the meeting has changed, we will be discussing all aspects of vascular endothelium.

There is no charge for the meeting. There will be a lunchtime buffet and refreshments at the end of the meeting.

Yours sincerely,



GUY NEILD
Professor of Nephrology

P.S. Please let other members of your Department know the date and venue.

XIII th INTERNATIONAL CONGRESS OF NEPHROLOGY

MADRID, SPAIN JULY 2-6 1995

Madrid, May 16, 1995

Mukhtar, A
College of Health Sciences

P.O. Box. 12
Manama

Bahrain

COPY

Dear Dr. Mukhtar, A

RE: Abstract # 9

TITLE: HLA -A, -B AND -C ANTIGENS AMONGST BAHRAINIS

We are happy to advise you that your abstract has been selected by the Program Committee for Poster Presentation at the XIIIth International Congress of Nephrology as detailed below.

Theme: Transplantation
Session Name: The major histocompatibility complex and tissue matching
Date: July 3, 1995

The number of your poster will correspond with the number printed in the program. Your poster board area is 2m wide and 0.95m high.

Please see the attached instruction form for preparing your poster and plan to mount your materials on your assigned board in the poster exhibit areas of the Palacio de Congresos at 09:00 hrs. and dismantle it between 18:00 and 19:00 hrs. on your exhibit date. You and/or you co-authors are expected to be present at your poster from 13:30 to 15:00 hrs. on Monday 3, Wednesday 5 and Thursday 6 and from 14:15 to 15:30 hrs. on Tuesday 4.

REGISTRATION

Please note that the early registration deadline is February 28, 1995, however, this deadline will be extended until March 15th exclusively for all the authors of abstracts to allow for possible postal delays. All authors who plan to attend must register for the meeting. Authors who have not registered will not be included in the Final Programme. Please contact Grupo Geyseco should you require further information.

Congratulations on having your abstract selected for the 1995 ICN Meeting.

Sincerely

L. Hernando
President XIII ICN 95

P. Aljama
President of the Selection Committee

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8th May 1995

Dear. Dr. Mukhtar,

Thank you for sending your paper "Effect of Cyclohexamide, Tunicamycin and Actinomycin D on MP 84 Expression by Mesangial Cells" for publication.

We have the pleasure of informing you that this paper will be published in the July-September '95 issue of Hamdard Medicus (Vol.XXXVIII, No.3).

With kind regards,

Yours sincerely,

Hakim Mohammed Said
Editor

Dr. Ahmed Mukhtar,
College of Health Sciences,
P.O. Box 12,
Bahrain.

ZB/JY.