

Myogenic Potential of Fetal Mesenchymal Stem Cells

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Introduction

Genetic diseases with intrauterine onset can lead to permanent neurological or musculoskeletal damage in early life. To prevent this, stem cells have been proposed as vehicles for fetal gene or protein delivery because of their ability to expand and give rise to large numbers of multipotent cells. Transplantation in early intrauterine life should capitalise on the ontological opportunity afforded by developing tissues, fetal immune naiveté and the endogenous seeding and engraftment of native stem/progenitor cells, thus raising the possibility that intrauterine therapy may eventually be advantageous for genetic diseases of postnatal onset.¹

Mesenchymal stem cells (MSCs): MSCs represent an attractive alternative for fetal therapy. MSCs from adult bone marrow^{2,3} have been well characterised and are under intensive investigation for therapeutic applications. They divide rapidly and are readily transducible with integrating vectors, maintaining transgene expression in-vitro and in-vivo without affecting multipotentiality.^{4,5}

Human fetal MSC (hfMSC): Human fetal blood contains a population of adherent cells with an immunophenotype comparable to adult MSCs. They proliferate more readily than adult MSCs,⁶ self-renew over 20 to 40 passages, differentiate along multiple mesenchymal lineages (Fig. 1) and have greater potential and differentiate into oligodendrocytes and haemopoietic cells (unpublished data). They circulate only in the first trimester, support haemopoiesis in co-culture and are similar to comparable populations in fetal liver and bone marrow, suggesting they are stromal cells migrating to sites of definitive haemopoiesis.⁷ hfMSCs were transplanted *in utero* at 65 days' gestation in a xenogeneic fetal sheep model to find engraftment in multiple organs, where they underwent site-specific tissue differentiation.⁸

Duchenne muscular dystrophy (DMD): DMD is a progressive X-linked myopathy affecting 1 in 3500 boys.⁹ Muscle wasting is apparent at 3 to 5 years, with affected individuals wheelchair-bound at 12 years and dying in the third decade of life from respiratory failure or cardiomyopathy.¹⁰ In most cases, deletions cause a premature stop codon in the dystrophin gene on chromosome Xp21, which are inherited in two-thirds of cases.¹¹ The genetic defect leads to near absence (<3%) of dystrophin, which otherwise protects muscle from contraction-related damage.¹² There is no effective treatment and myoblast transplants have proven disappointing.¹³

Stem cell therapy for DMD: Several lines of evidence suggest that MSCs participate in regeneration due to muscle damage. Ferrari reported low-level uptake in injured muscle of intravenously administered allogenic bone marrow cells in immunodeficient mice.¹⁴ In mdx¹⁵ mice (a model of dystrophin deficiency) transplanted after lethal irradiation with adult bone marrow cells, Gussoni found dystrophin in up to 10% of muscle fibres. Bittner showed the presence of occasional bone marrow-derived cells in both skeletal

and cardiac muscles in mdx mice.¹⁶ Furthermore, a case report of a boy with DMD and a prior bone marrow transplant for SCID documents long-term persistence of donor nuclei in muscle.¹⁷ Although the responsible cell type is not known, it is likely that these are MSCs, based on Ferrari's finding of greater engraftment of adherent compared to non-adherent marrow cells, and Gussoni's higher levels of dystrophin expression after transplantation with non-purified marrow compared to marrow enriched for HSC. More recently, De Bari transplanted adult human synovial membrane MSCs into both an injury and mdx mouse model to find incorporation into myofibres and restoration of dystrophin expression, respectively.¹⁸ The utility of a fetal to fetal approach is supported by mid-gestation transplants in mdx mice with unpurified fetal liver or adult bone marrow, which resulted in widespread engraftment and myogenic differentiation.¹⁹

We aimed to study myogenic differentiation of hfMSC in-vitro and in-vivo. This would define the conditions whereby myogenesis occurs and may allow the use of lineage committed progenitors in transplantation strategies.

Materials and Methods

hfMSC Samples

After informed consent, first-trimester fetal blood samples were obtained by ultrasound-guided cardiac aspiration between 7 and 13 weeks' gestation at surgical termination of pregnancy. Fetal blood was plated at a density of 10⁵/mL and cultured in 10% fetal bovine serum (Stem Cells, Vancouver). Upon reaching confluence, cells were trypsinised and passaged for cryopreservation or used in the following experiments.

In vitro Myogenic Differentiation

hfMSCs were plated at high densities in fibronectin-coated chamber slides and cultured in a variety of conditions known to promote myogenesis in murine bone marrow stromal stem cells.

1. 5-azacytidine²⁰ (Sigma, UK), a hypomethylation agent, was used as an induction agent at varying concentrations in serum-free conditions.
2. Myoblast-conditioned media²¹ was used at various concentrations in both serum-containing and serum-free media supplemented with additional growth factors (PDGF-BB and EGF, Sigma, UK).

Cells were fixed in methanol/acetone and incubated with monoclonal antibodies to desmin (DAKO, UK) and MF20 (sarcomeric myosin subunit – hybridoma line). Briefly, slides were washed and blocked with 5% albumin and goat serum, and developed using a streptavidin-biotin alkaline phosphatase reaction or a fluorochrome label.

In vivo Work

Immunodeficient adult mouse injections: In order to demonstrate *in*

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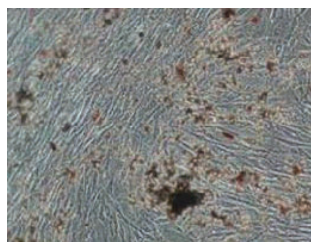


Fig. 1a. Osteogenic differentiation-Von Kossa staining of calcium hydroxyapatite crystals.

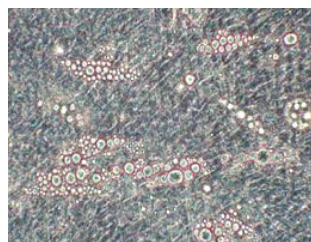


Fig. 1b. Adipogenic differentiation-distinct oil droplets after 21 days in culture.

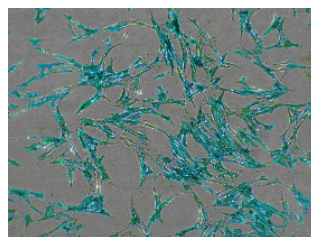


Fig. 1c. X-Gal staining after retroviral transduction with LacZ.

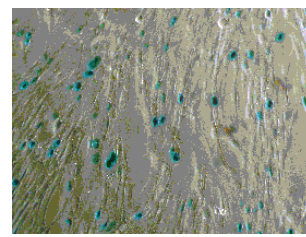


Fig. 1d. X-Gal staining of hfMSCs after HIV-1 lentiviral transduction with nuclear localising LacZ.

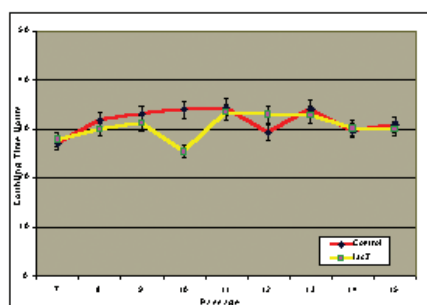
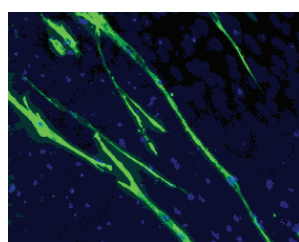
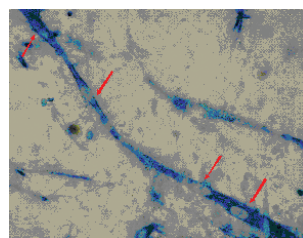


Fig. 1e. Growth kinetics of transduced (yellow) versus mock transduced (red) MSC over successive passages.

Fig. 1. hfMSCs – differentiation and viral transduction.



MF20-FITC staining at D12 myogenic differentiation showing multi-nucleated fibres staining positive for MF20 amidst negative cells.



Desmin staining of hfMSCs at D14 demonstrating multi-nucleated fibre.

Fig. 2. In vitro myogenic differentiation – MF20 and Desmin Staining.

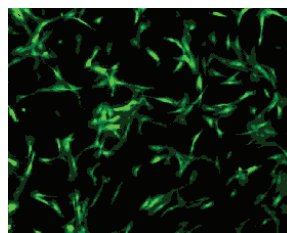


Fig. 1f. GFP expression after lentiviral transduction.

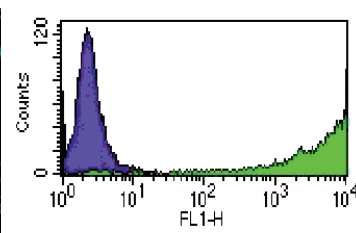


Fig. 1g. FACS data showing transduced (green) versus mock transduced (purple) cells with GFP fluorescence.

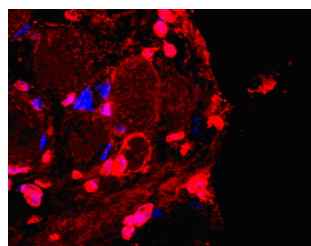


Fig. 3. In vivo adult mice transplants.

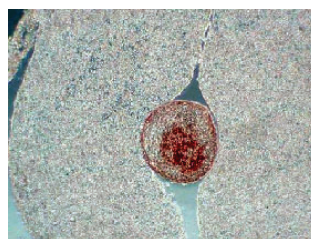


Fig. 4. In utero transplanted.

vivo myogenesis, 5×10^5 low passage hfMSCs were injected into irradiated tibialis anterior muscles of immunodeficient c- γ -RAG2 mice.²² Irradiation incapacitates host myogenic cells and possibly releases growth factors,^{23,24} resulting in increased muscle formation from implanted cells.²² After 28 days, the muscle was harvested, snap frozen and sectioned with a cryostat. Sections were blocked with rabbit serum and papain-digested whole fragments of unlabelled secondary anti-mouse immunoglobulins,²⁵ were stained with mouse monoclonal anti-lamin A/C (human nuclear envelope – Novocastra, UK) and human spectrin (human muscle sarcolemmal protein – Novocastra, UK) antibodies, and visualised with a streptavidin-biotin-alexa fluorochrome.

In utero transplantation into MF1 mouse: As a preliminary to *in utero* transplantation work with mdx mice, 5×10^5 hfMSCs were transplanted *in utero* into immunocompetent MF1(non-dystrophic) fetal mice. Two days later, they were harvested, formalin fixed, wax-embedded and prepared in 5 μ m sections. Human cells were sought

via immunostaining with a human vimentin (DAKO, UK) and visualised using a peroxidase system (Vector Lab, UK).

Transduction

The retrovirus pBMN-Z-I-Neo expressing LacZ (G. Nolan, Stanford) was transiently transfected into 293T producer cells (ATCC-SD3443) utilising CaPO₄ precipitation.²⁶ Two days after transfection, retroviral supernatant was harvested used for transduction experiments. Early-passages MSCs were plated overnight at low densities 5×10^3 /cm² and subjected to a 3-cycle infection regime. Incubation with retroviral supernatant and polybrene (4 μ g/mL) for a total of 6 hours per cycle was performed. G418 selection and analysis of transgene expression (β -galactosidase) by X-Gal staining was done. Growth kinetics and differentiation potential at short- and long-term time points were performed. Osteogenic and adipogenic differentiation was achieved with standard protocols as published elsewhere.⁷

Because retroviral gene delivery is inefficient, requiring drug

selection,²⁷ multiple rounds of transduction⁴ and prone to expression shutdown,^{27,28} a VSV-G pseudotyped lentiviral vector RRL.ppt.hPGK.GFP.pre.sin-18 (gift from K Brimah and G Cossu) was prepared using transient co-infection with a helper and env plasmid system to 293T producer cells. A single-round infection with polybrene was performed. FACS analysis was done at 48 hours to determine eGFP expression.

Results

In vitro Myogenesis

Unlike murine MSC, chemical induction with a varying concentration of 5-azacytidine for differing periods of time did not promote myogenic differentiation, but led to massive cell death of hfMSCs.

Culturing hfMSCs with myoblast-conditioned media led to the development of desmin-positive cells by day 3 and MF20 positive cells by day 7. By Day 14, large multi-nucleated muscle fibres can be seen that stain positively for both desmin and MF20, indicative of muscle differentiation (Fig. 2).

In vivo Myogenesis

After transplantation into immunodeficient damaged murine muscle, hfMSC participated in regeneration of muscle by forming spectrin positive muscle fibres, albeit at low levels (<1%) – 5 to 6 fibres per muscle section (Fig. 3).

Two days after *in utero* transplantation into immunocompetent MF1 strains, hfMSCs can be detected by staining for human vimentin (Fig. 4).

Transduction

Transduction efficiencies of 44.1% to 48.5% were achieved with the retroviral vector. After G418 selection, the short-term growth kinetics and osteogenic, adipogenic and myogenic differentiation potential remains similar to mock infected control hfMSCs (Fig. 1). On the other hand, lentiviral transduction reached 100% efficiencies as demonstrated by FACS and microscopy.

Discussion

We have shown that hfMSCs can undergo myogenic differentiation under permissive conditions in-vitro, and in an immunodeficient mouse model form muscle, in response to environmental cues, albeit at low levels. However, this mouse model has only low levels of muscle regeneration compared to the dystrophic mouse (mdx) which approximates human DMD. Hence, there is little selective pressure for the transplanted cells.

Identification of human cells two days after injection into E14 immunocompetent MF1 mice provides proof-in-principle that they survive in the xenogenic fetal environment. Analysis of post injected mice at longer time intervals in the near future will allow us to determine the site(s) of engraftment, particularly whether they contribute to muscle regeneration in dystrophic mdx mice. hfMSCs are readily transducible with high efficiencies using a lentiviral construct.

This work evaluates a novel type of MSCs for allogeneic or even autologous fetal therapy of not only debilitating childhood muscle diseases, such as muscular dystrophy, but a range of other mesenchymal deficiency conditions as well. With advances in early fetal blood sampling techniques, *in utero* treatment of genetic diseases can be treated with genetic manipulation of autologous hfMSCs.

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Subjective versus Observer-rated Insight in First-Episode Psychosis in Singapore

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Introduction

Many patients with psychotic disorders lack insight into their illness and this has been suggested to be a manifestation of the illness itself, rather than a coping strategy.¹ Most of the studies on insight have focused on the clinical correlates of poor insight, such as psychopathology,²⁻⁴ neuropsychological impairment^{2,5} and structural brain damage.⁶ Studies have reported an association between lack of insight and a poor outcome of the illness,^{7,8} as well as with non-compliance with treatment.⁹⁻¹¹

Insight is viewed as a complex and multi-dimensional phenomenon that includes elements of a psychological, psychopathological, neurocognitive and interactional nature.³ However, results from previous studies on insight have been largely inconclusive. This was documented in a meta-analysis⁴ of 40 published English language studies to determine the magnitude and direction of the relationship, or effect size, between insight and symptom domains in schizophrenia, and to determine moderator variables that were associated with the variations in effect sizes across studies. Limitations have been attributed to difficulties with defining and measuring levels of insight in this population.¹² Insight is usually assessed with rating instruments, which may be either a clinician or an observer-rated insight scale,¹³ or a self-rated scale.¹⁴ The latter is not so extensively used, possibly because these self-rated instruments are assumed to be incapable of adequately capturing how patients understand and feel about their illness.¹⁵ However, studies have shown that patients with psychosis are able to use a range of different frameworks to understand and explain their experiences.¹⁶

To date, there has been no study to compare and elicit the differences (if any) between subjectively reported insight and objectively reported insight. The present study aimed to study this relationship in Asian patients with first-episode psychosis.

Materials and Methods

Sample

The study population comprised 98 consecutive patients between the ages of 15 and 40 with first-episode psychosis and who are able to read and write English. Individuals with current substance abuse, mental retardation, neurological disorder or any other medical illness were excluded.

Approval for the present study was obtained from the Research and Ethics Committee of the Institute of Mental Health. All patients provided written informed consent.

Procedure and Measures

The diagnosis was established using the Structured Clinical Interview for DSM-III-R (Patient Version) (SCID-P).¹⁷ The Duration of Untreated Psychosis (DUP) was defined as the time interval between the onset of psychotic symptoms (hallucinations, delusions, thought disorder and/or disorganised behaviour) and hospitalisation for psychosis or initiation of adequate treatment.¹⁸

The severity of psychopathology was assessed with Positive and

Negative Syndrome Scale for Schizophrenia (PANSS),¹⁹ and the quality of life was assessed with the World Health Organization Quality of Life-Bref (WHO-QoL BREF).²⁰ This is a 26-item self-administered subjective measure of quality of life. Its multidimensional nature allows for 4 domains of assessment: physical health, psychological functioning, social relationships and environment. High scores indicate good QoL.

Insight was assessed with the Birchwood Insight Scale¹⁴ which is an 8-item self-report measure of subjective insight based on the awareness of illness, need for treatment and attribution of symptoms. Scores range from 0 to 12, with low scores indicating reduced insight. All the patients were also assessed by a research psychologist with the Scales to Assess Unawareness of Mental Disorder (SUMD).^{7,13} This scale assesses the awareness of the illness, response to medication, social consequences of having a mental disorder, and specific symptoms of the illness. High scores on this scale indicate less insight. Interrater reliability for the PANSS and the SUMD was >0.8.

Statistical analyses were carried out using SPSS 11.0. Normality assumption of quantitative data was checked using Kolmogorov

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF STUDY PATIENTS (n = 98)

Variable	No. of patients (%)	
Gender		
Male	57	(58.2)
Female	41	(41.8)
Race		
Chinese	68	(69.4)
Malay	23	(23.5)
Indian	6	(6.1)
Others	1	(1.0)
Religion		
Buddhism/Taoism	47	(47.9)
Christianity	13	(13.3)
Islam	24	(24.5)
Hinduism	2	(2.0)
No religion	12	(12.2)
Marital status		
Single	66	(67.3)
Married	24	(24.5)
Divorced/Separated	7	(7.2)
Widowed	1	(1.0)
Education		
Primary	14	(14.4)
Secondary	38	(38.7)
Tertiary	30	(30.6)
Vocational institute	10	(10.2)
Pre-university	6	(6.1)
Living situation		
Alone	7	(7.1)
With husband/wife	25	(25.6)
With parents/siblings	66	(67.3)
Employment		
Employed	26	(26.6)
Unemployed	42	(42.9)
Student	12	(12.2)
Homemaker	12	(12.2)
National service	6	(6.1)

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TABLE II: CORRELATIONS BETWEEN SUBJECTIVE INSIGHT (BIRCHWOOD INSIGHT SCALE) AND QUALITY OF LIFE (WHO-QoL)

	WHO-QoL domains				
	Physical Health	Psychological Health	Social Relationship	Environmental Health	Total QoL score
Birchwood Insight domains					
Relabel symptoms	-.278**	-.283**	-.300**	-.255*	-.314**
Awareness of illness	-.229*	-.208*	-.183	-.186	-.248*
Need for treatment	-.227*	-.061	-.042	-.112	-.148
Total Birchwood score	-.330**	-.257*	-.247*	-.253*	-.325**

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Smirnov 1 sample test. Pearson's correlation coefficients were calculated to study the relationship between objective and subjective measures of insight if normality assumptions were satisfied otherwise Spearman's correlation will be presented. A multiple regression was performed on the total WHO-QoL score using age, gender, ethnic group (Chinese vs non-chinese), education (tertiary vs non-tertiary), employed vs unemployed, living alone or not, DUP since onset of symptoms, total Birchwood score, total PANSS score and total SUMD score.

Results

The mean (SD) age of the 98 patients was 28.9 (7.1) years. The mean (SD) DUP was 21.5 (35.7) months; the median DUP was 3 months. Forty-two (42.9%) of the 98 patients met DSM-IV criteria for schizophrenia, 24 (24.5%) for schizophreniform disorder, 4 (4.1%) for schizoaffective disorder, 12 (12.2%) for brief psychotic disorder, 7 (7.1%) for psychotic disorder not otherwise specified and 9 (9.2%) for mood disorders with psychotic features. Demographic characteristics are described in Table I.

There was no significant relation between objectively-rated illness awareness with subjective measures of insight ($r = -0.094$, $p = 0.361$; Spearman's correlation) (Fig. 1).

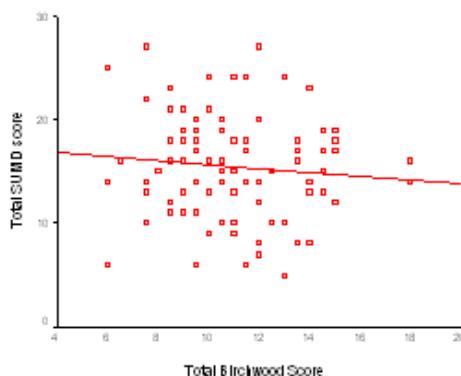


Fig. 1. Scatter plot of total SUMD score against total Birchwood score.

None of the objective and subjective insight items were found to be associated with psychopathology. Significant relationships were found between domain scores of the Birchwood Insight Scale and the WHO-QoL (correlation coefficients are presented in Table II).

The following predictors, age, gender, ethnic group (Chinese vs non-chinese), education (tertiary vs non-tertiary), employed vs unemployed, living alone or not, DUP since onset of symptoms, total Birchwood score, total PANSS score and total SUMD score, were modeled in a multiple regression analysis on total WHO-QoL. Only the total Birchwood score was significant with every unit increase in Birchwood score, the WHO-QoL decreases by 2 units (95% CI 0.6 to 3.6, $p = 0.007$).

Discussion

Our findings suggest that there is no agreement between subjectively rated insight and the observer-rated insight. As insight has been conceptualised as a multi-dimensional construct,³ the two instruments may actually be measuring different dimensions of insight. As the symptomatology of schizophrenia is also multi-dimensional, it is not surprising that we did not find a significant association between insight and psychopathology, as the instruments used in our study may be relatively simple and may not capture the complexity of insight and psychopathology of schizophrenia. This is consistent with the findings of previous studies,^{7,10,11} which reported no significant association between insight, acute psychopathology and negative symptoms. This has led to the suggestion that further studies should examine how specific dimensions of insight relate to other symptoms in psychotic illnesses.⁴

A limitation of our study is that we did not assess the level of neurocognitive functioning in this group of patients. The neuropsychological view of insight is that awareness of illness may be a higher-order cognitive ability,⁶ and previous studies have hypothesised that poor insight in psychosis could largely be a function of impaired information processing capacities.^{2,6}

Although we have included only English-speaking patients, there is also a need to validate these instruments in our local population as cultural factors would determine the perception of the presence of psychiatric symptoms, and the reporting of these symptoms may also vary from culture to culture.²²

Our findings suggest that objective insight ratings alone may be insufficient to adequately account for the level of awareness that a patient has about the psychotic illness. As such, future studies should incorporate different scales measuring different dimensions in the assessment of insight, and take into consideration the cultural nuances of the study population.

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Young Investigator's Award Finalist

Usefulness of Serum Soluble CD38 and CD157 Levels in Differentiating SLE, RA and Healthy Adults and their Relationship with Disease Activity

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Introduction

Human CD38 is a transmembrane glycoprotein involved in lymphocyte activation and adhesion to endothelium. The ectocellular domain of the molecule possesses properties of a bi-functional enzyme catalysing both the synthesis from NAD⁺ and the hydrolysis of the calcium-releasing metabolite cyclic ADP-ribose (cADPR).¹⁻³ In addition to the existence of a transmembrane CD38, a soluble form of CD38 (sCD38) is detectable in the cell culture supernatant of allo-activated T lymphocytes and of several tumor cell lines and also *in vivo*. It is assayable in normal (fetal serum and amniotic fluid) and pathological (serum and ascites from patients with multiple myeloma, and serum from patients with AIDS) biological fluids.⁴

Human CD157 is a glycosylphosphatidylinositol-anchored membrane protein, with ADP-ribosyl cyclase/cADPR hydrolase activity, having 30% homology with CD38. The expression patterns of both molecules are overlapping in several tissues and haemopoietic systems, suggesting that CD157 and CD38 are functionally co-operating or compensating in certain situations.⁵ Bone marrow nurse-like cells in patients with rheumatoid arthritis (RA), which express greater amount of CD157, were demonstrated to be able to rescue B-cells from spontaneous apoptosis.⁶ Similarly, soluble CD157 (sCD157) has also been noted in the serum of patients with RA.⁷ It was proposed that very high level of sCD157 might signify severe RA disease in RA.

Objectives

In inflammatory conditions or autoimmune diseases, soluble forms of membrane proteins have been detected in sera and clinical significance has been reported.^{8,9} We aimed to measure serum sCD38 and sCD157 in patients with RA and systemic lupus erythematosus (SLE), and to identify their association with disease activity.

Materials and Methods

Serum samples were obtained from 120 patients with SLE and 90 patients with RA, who were consecutively seen in the outpatient rheumatology clinics between December 2000 and May 2001, after informed consent. All RA and SLE patients fulfilled the American College of Rheumatology criteria for RA¹⁰ and SLE¹¹ respectively. Control serum samples were obtained from 56 consented healthy donors. Disease activity was assessed using Disease Activity Score using 28-joint count (DAS 28) and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) for RA and SLE, respectively. Disability in RA was assessed using modified Health Assessment Questionnaire (mHAQ).

sCD38 and sCD157 levels were measured using enzyme-linked immunosorbent assay (ELISA), which utilised specific monoclonal antibodies for the identification of molecules of interest. The levels were expressed in optical density units (ODU).

Continuous data are presented in the form of mean $\pm$ standard

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deviation or median and range. Categorical data is presented as percentage. Analysis of the data was done using the Inter-cooled STATA 7.0 for Window. Kruskal-Wallis test, Mann Whitney U test, and Spearman's rank correlation were performed as indicated. Significance level was set at 0.05. Receiver operating characteristic (ROC) analysis was used to assess the discriminating power of sCD38 and sCD157 at various levels.

Results

A total of 90 RA and 120 SLE patients were recruited. Mean age of RA and SLE patients was 49.1 ± 10.7 years and 37.2 ± 11.3 years, respectively. Female constituted 85.0% and 93.4% of RA and SLE patients, respectively. Chinese formed 77.4% of RA patients and 76.9% of SLE patients. The mean disease duration was 4.7 ± 4.6 years in RA (median 3.5 years) and 5.9 ± 5.0 years in SLE (median 5 years). The DAS 28 score of RA patients was 3.7 ± 1.3 . SLE patients had a median SELDAI of 4 (range 0 to 24).

sCD38 levels were significantly different among SLE, RA and healthy donors, with the levels being lowest in SLE and highest in healthy donors (0.074 ± 0.043 ODU for SLE versus 0.122 ± 0.044 ODU for RA versus 0.157 ± 0.005 ODU, $p < 0.0001$).

sCD157 was undetectable in 4% of healthy controls, 21% of RA and 5% of SLE ($p < 0.0001$). Furthermore, sCD157 levels in RA identified a subgroup of patients with very high levels of sCD157 (35,000 ODU in 23% of cases; mean levels: $35,280 \pm 220$ ODU) compared to the rest of the group ($14,376 \pm 1317$ ODU). None in normal healthy controls and only 2 patients with SLE had such high levels. However, the disease activity as assessed using DAS 28 among those with high levels did not differ significantly from the rest (3.7 ± 1.7 and 3.6 ± 1.4 , respectively). Similarly, median mHAQ score did not show significant differences between the 2 groups (0.125 versus 0.5).

Discussion

Our study showed significantly suppressed sCD38 in both SLE and RA relative to the levels in healthy adults. This was initially surprising since activated T and B cells in these 2 autoimmune diseases are well known and membrane surface CD38 expression is up-regulated in activated lymphocytes.¹² The results of our other study (unpublished data), which showed increased production of autoantibody to CD38 offered a possible explanation to the suppressed sCD38 levels found. In parallel with endocytosis, shedding of CD38 is a regulatory element in activation and adhesion processes mediated by CD38. Internalisation of CD38 is triggered off by both agonistic and non-agonistic monoclonal antibodies.¹³ With the elevated anti-CD38 antibody production and, thus, internalisation process, reduced shedding of sCD38 is expected in SLE and RA. This further supported by the fact that in SLE, where the production of anti-CD38 autoantibody is highest (unpublished data), sCD38 level is the lowest. The difference in sCD38 levels in various conditions allows us to differentiate healthy adult, RA and SLE patients, as shown with ROC analysis. This differentiation is especially useful in situation where SLE presented as rheumatoid-like arthritis or in RA with positive ANA.

We also confirmed the previous finding of the elevated sCD157 levels in RA with a subgroup of patients with extremely high levels

and its lack of relationship with the disease activity as assessed using DAS 28.⁷ However, different from the previous report, we did not find very high level of sCD157 among those with severe disease as determined by disability. Furthermore, in our study, SLE had comparably high level as in RA, even after those with very high levels (≥ 35000 ODU) were excluded. It is recognised now that B-cells also play an important pathogenetic role in RA, as in SLE. Our finding is, thus, not surprising and might suggest that sCD157, as a molecule which salvages B-cells from apoptosis,⁵ plays an important role in the pathogenesis of both diseases.

Conclusion

Suppressed serum sCD38 levels and raised serum sCD157 levels were found in RA and SLE. The levels of sCD38 may be helpful in differentiating SLE, RA and the healthy state. sCD38 levels correlate significantly with disease activity in RA, but not in SLE. High level of sCD157 is more likely to be found in RA. Our study did not find any relationship between disease activity in RA and sCD157 level.

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