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Session 2: Micronutrients and the immune system
Role of selenium-containing proteins in T-cell and macrophage function

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Selenium (Se) has been known for many years to have played a role in boosting the immune function, but the manner in which this element acts at the molecular level in host defence and inflammatory diseases is poorly understood. To elucidate the role of Se-containing proteins in the immune function, we knocked out the expression of this protein class in T-cells or macrophages of mice by targeting the removal of the selenocysteine tRNA gene using loxP-Cre technology. Mice with selenoprotein-less T-cells manifested reduced pools of mature and functional T-cells in lymphoid tissues and an impairment in T-cell-dependent antibody responses. Furthermore, selenoprotein deficiency in T-cells led to an inability of these cells to suppress reactive oxygen species production, which in turn affected their ability to proliferate in response to T-cell receptor stimulation. Selenoprotein-less macrophages, on the other hand, manifested mostly normal inflammatory responses, but this deficiency resulted in an altered regulation in extracellular matrix-related gene expression and a diminished migration of macrophages in a protein gel matrix. These observations provided novel insights into the role of selenoproteins in the immune function and tissue homeostasis.

Macrophage: Selenium: Selenocysteine: T-cells

Roles of selenium in immune function

Selenium (Se) is an important micronutrient in the diet of human individuals and other mammals. It is known to serve a wide variety of functions in health and development including roles in cancer and heart disease prevention, viral inhibition, male reproduction and the immune function, delaying the ageing process and the onset of AIDS in HIV positive patients[1]. Interestingly, of all these health benefits attributed to Se, a role of this element in boosting the immune function was amongst the first indications that Se, which incidentally was long thought to be a toxin[2], served as an advantageous agent in health. That is, only 2 years after Se was shown to prevent liver necrosis in rats, which provided the first demonstration that this element was essential in the diet of mammals[3], McConnell injected ^{75}Se into dogs and found that the isotope was incorporated in leucocytes[4]. Since this early finding, numerous studies have suggested roles of Se in both the adaptive and innate immune systems[5–15]. The mechanism of how Se acts at the molecular level in boosting the immune system is only beginning to be understood, primarily through the action of selenoproteins[16,17]. The possible action of small molecular weight selenocompounds in enhancing immune activity has not been examined. The focus of this paper is on the role of selenoproteins in the immune system. Several excellent reviews on Se in the immune system have recently been published.
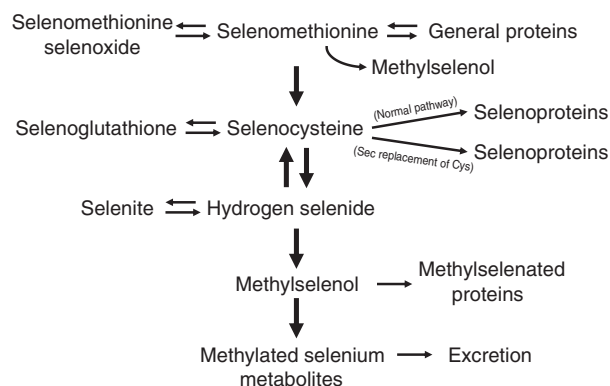
Fig. 1. Se and selenomethionine metabolism in mammals. Pathways of Se utilization in mammals are shown. Further discussion of Se and selenomethionine metabolism may be found elsewhere[40,41]. Sec, selenocysteine (see text); Cys, cysteine.

They provide an in-depth analysis of wide range benefits that Se offers in the immune function[16–18].

Selenium metabolism in mammals

The possible means of how selenium is metabolized in mammalian systems is shown in Fig. 1. Se can potentially make its way into protein by multiple pathways, but only one of these pathways is used to specifically incorporate this element into selenoproteins: through the Se-containing amino acid, selenocysteine (Sec), the 21st amino acid in the genetic code[19]. Sec is programmed to be inserted into protein by the stop codon, UGA, and thus, this codeword serves as a dual function in mammals. Its biosynthesis occurs by a novel pathway in eukaryotes in that the synthesis is carried out on Sec tRNA, designated Sec tRNA[Ser]Sec and proceeds as follows: (1) serine is attached to tRNA[Ser]Sec in the presence of seryl-tRNA synthetase and ATP to form Ser-tRNA[Ser]Sec; (2) the seryl moiety is phosphorylated in the presence of O-phosphoseryl-tRNA[Ser]Sec kinase and ATP to form O-phosphoseryl-tRNA[Ser]Sec and (3) O-phosphoseryl-tRNA[Ser]Sec in turn serves as a substrate for Sec synthase. In this reaction, monoselenophosphate, which is the active donor of Se, donates Se to the acceptor intermediate generated by Sec synthase to yield selenocysteyl-tRNA[Ser]Sec. Monoselenophosphate is synthesized by selenophosphate synthetase 2 from selenide (or other Se compounds) and ATP. The details of Sec biosynthesis have been reviewed elsewhere[20]. The other pathways wherein Se can be non-specifically incorporated into protein, namely by selenomethionine replacing methionine and Sec replacing cysteine, may result in deleterious effects to the cell, especially if such insertion occurs to any appreciable extent.

Roles of selenoproteins in the immune function

In our studies examining the role of selenoproteins in T-cell development, we have taken advantage of the fact that selenoprotein synthesis is dependent on Sec tRNA which is responsible for inserting this Se-containing amino acid into protein. By targeting the removal of the floxed Sec tRNA gene, designated *Trsp*, in T-cells or macrophage

of mice by *Cre*-recombinase under control of the *Lck* promoter or the lysozyme M promoter, we generated selenoprotein-deficient T-cells[21] or selenoprotein-deficient macrophage[22], respectively. The expression of selenoproteins in these two cell types and the consequences of the loss of selenoprotein expression in these cell types on immunity are discussed later. Another study involving the targeted removal of *Trsp* in macrophages and simultaneously *Nrf2*, a transcription factor that regulates antioxidant enzyme expression, demonstrated that knockout mice manifested a reduction in viability and an increase in oxidative stress and susceptibility to hydrogen peroxide compared to mice in which either of the two genes was knocked out[23]. Mice in which only *Trsp* was targeted for removal of macrophage had an increase in the gene expression involving oxidative stress and detoxification enzymes. These results, along with several lines of clinical evidence mentioned above prompted us to examine whether selenoproteins link redox regulation to immunity and inflammation in mammals.
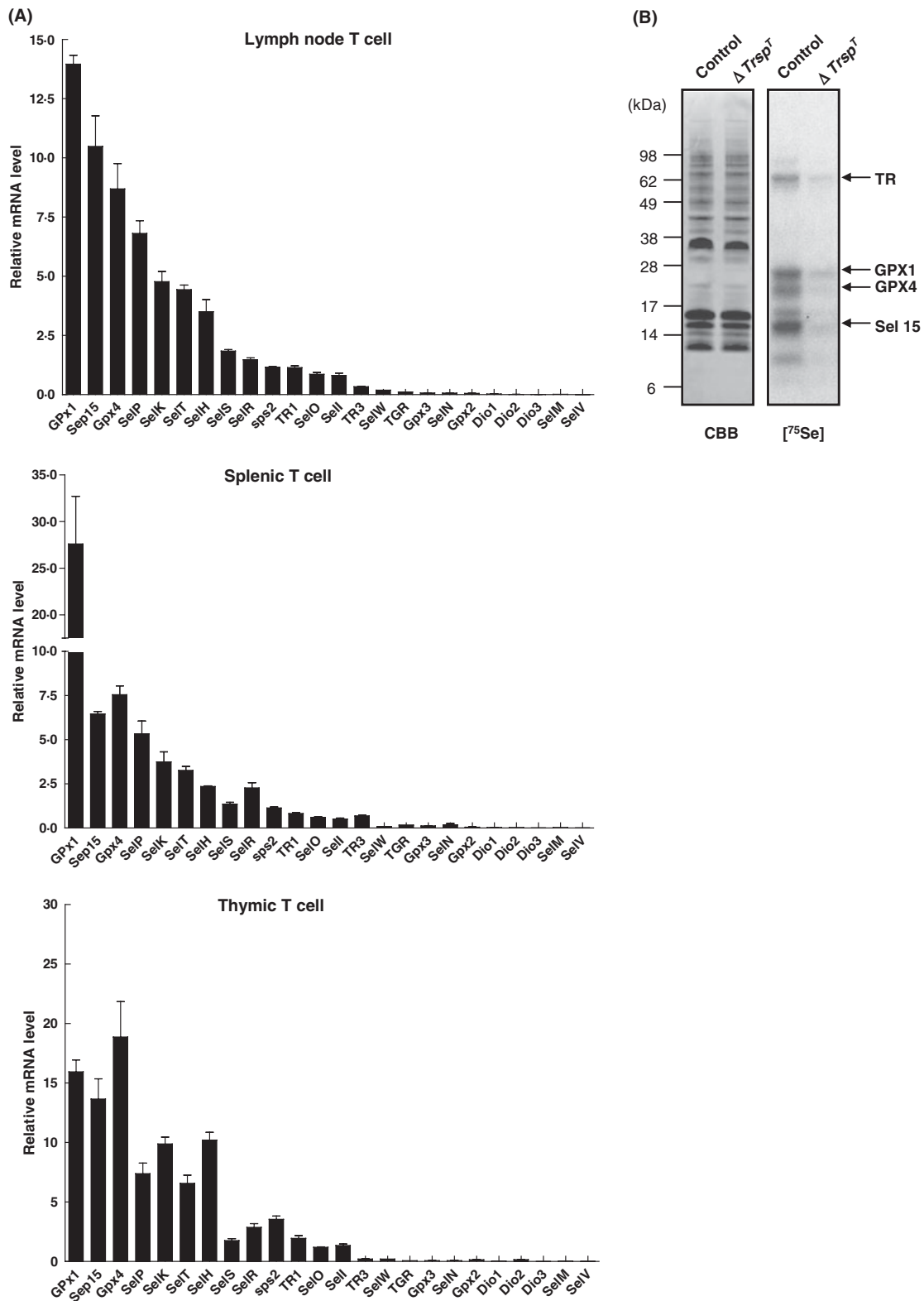
Selenoproteins in T-cells

mRNA expression and T-cell development

There are 24 selenoprotein genes in mice[24] and individual selenoproteins and selenoprotein mRNA manifest tissue specificity in their patterns of expression and in the manner in which their expression is regulated[25]. We examined the expression of the mRNA levels transcribed from each of the 24 selenoprotein genes by isolating total RNA from the three lymphoid organs, lymph nodes, spleen and thymus of normal mice and analysing the expression by real-time PCR. As shown in Fig. 2(A), GPx1 mRNA was highly expressed in all three organs. Several other selenoprotein mRNA, namely Sep15, GPx4, SelP, SelK, SelT and SelH, were moderately-to-highly expressed in each organ. Several additional selenoprotein mRNA were also made, while others were only slightly, or not at all, detectable. It is not clear why there is some variation in the levels of selenoprotein mRNA expression between the three tissues.

We also examined selenoprotein expression in T-cells by labelling control and selenoprotein deficient ($\Delta Trsp^T$) mice with ^{75}Se, isolating the T-cells from the thymus and examining the labelled selenoproteins by gel electrophoresis (Fig. 2(B)). Several selenoproteins were labelled in control mice, some of which have been identified in earlier studies[26]. Virtually no selenoproteins were labelled in the $\Delta Trsp^T$ T-cells and the faint labelling is most likely due to contamination from other cell types[27].

The thymus, spleen and lymph nodes of the affected mice manifested moderate-to-severe atrophy wherein their mass and cellularity were decreased to varying degrees ranging between 50% and 80% of those of control mice. The T-cell population in lymphoid organs, which was analysed by flow cytometry, was altered significantly. For example, the CD3$^+$ population in splenocytes of $\Delta Trsp^T$ mice was about 50% of that of control splenocytes, while the CD8$^+$ T-cell sub-population was more substantially reduced (>50%). Although the consequences of the partial loss of functional T-cells and in particular CD8$^+$ T-cells,

Fig. 2. Selenoprotein expression in T-cells. (A) Expression of selenoprotein mRNA was assessed by quantitative real-time PCR. Purified T-cells were obtained from lymph nodes, spleen and thymus of mice as described[21]. (B) Expression of selenoproteins was assessed by ^{75}Se-labelling as described[27]. The identities of major labelled selenoproteins are designated on the right panel; and the left panel shows Coomassie Blue-stained (CBB) gel that was used as a loading control.

was not clearly defined in our study, other studies have shown that Se deficiency in the diet, which is known to cause a loss or reduction in selenoprotein function, impairs antiviral immunity and exacerbates viral pathogenesis[28,29].

T-cell activation

We next tested the ability of selenoprotein-deficient T-cells to be activated by T-cell receptor (TCR) signalling. Purified control T-cells and $\Delta Trsp^T$ T-cells were cultured in the presence or absence of anti-CD28 and anti-CD3 antibodies wherein the presence of these antibodies mimicked TCR activation and stimulated T-cells to proliferate[21]. The amount of ^{3}H-thymidine incorporated into both cell lines was measured after 60 h as an indication of the level of proliferation. Control cells incubated with both antibodies manifested a far more pronounced level of proliferation compared to unstimulated cells, whereas the level of proliferation of $\Delta Trsp^T$ T-cells was dramatically reduced under the same conditions (Fig. 3(A)). The data suggested that selenoprotein expression appeared to be essential for the ability of T-cells to proliferate in response to TCR stimulation.

Since TCR activation is known to produce IL-2 in T-cells and generate T-cell IL-2 receptor expression, we initially examined IL-2 production in control and $\Delta Trsp^T$ T-cells (Fig. 3(B))[21]. Both cell lines produced similar amounts of IL-2 in response to TCR activation. However, $\Delta Trsp^T$ T-cells manifested a much reduced IL-2 receptor induction compared to control cells (Fig. 3(C)).

Since TCR is coupled to multiple signalling pathways and it is known that mitogen-activated protein kinase extracellular signal-regulated kinase (ERK) links TCR activation to T-cell proliferation[30], we examined ERK activation in control and selenoprotein-deficient T-cells in response to stimulation with CD3/CD28 antibodies[21]. As shown in Fig. 3(D), T-cells from $\Delta Trsp^T$ mice manifested a very slight appearance of the phosphorylated or active forms of ERK following the treatment with anti-CD3 and anti-CD28 antibodies, while T-cells from control mice manifested a rapid appearance of the active forms of ERK.

The above data demonstrated that the loss of selenoprotein expression in T-cells resulted in defects in the development of functionally mature T-cells and their TCR-dependent activation. It is highly likely that such anomalies result in an impairment in T-cell-mediated immune responses. To further examine this possibility, control and $\Delta Trsp^T$ mice were immunized with NP-OVA ((4-hydroxy-3-nitrophenyl)acetyl-ovalbumin), an antigen that stimulates T-cell antibody production. Serum was taken from the immunized mice at different time periods after immunization and the levels of antigen-specific Ig were assessed by ELISA. Of the several Ig classes examined, IgM, IgG1, IgG2a, IgG2b and IgG3, the serum levels of antigen-specific antibodies were poorly raised in $\Delta Trsp^T$ mice (Fig. 3(E)). Thus, these data suggest that selenoproteins play an important role in T-cell immunity.

Reactive oxygen species in $\Delta Trsp^T$ T-cells

Since several selenoproteins are known to act as antioxidants and therefore have roles in protecting cells against

oxidative stress, we examined whether selenoprotein-deficient T-cells produce higher levels of reactive oxygen species (ROS) than the corresponding control cells and how this may relate to T-cell defects in $\Delta Trsp^T$ mice. To elucidate the basal and TCR-stimulated ROS generation in $\Delta Trsp^T$ and control cells, we labelled the cells with cell-permeable, oxidation-sensitive DCFDA (2′-7′-dichloro-fluorescin diacetate) dye. Interestingly, as shown in Fig. 4(A), the oxidation of DCFDA due to the generation of ROS was present to a greater extent in T-cells of $\Delta Trsp^T$ mice than in control mice even without TCR stimulation. TCR stimulation enhanced the ROS production in control cells, but had little effect on the ROS levels in $\Delta Trsp^T$ T-cells. The data suggest that selenoproteins have a major role in suppressing ROS production in T-cells and that the loss of this protein class results in T-cells being defective in TCR-induced and ROS-sensitive responses.

Finally, $\Delta Trsp^T$ T-cells were incubated in the presence of N-acetyl cysteine (NAC), which is known to block endogenously produced ROS, to assess whether this reagent can restore the ability of these cells to proliferate in response to TCR stimulation (Fig. 4B). The ability of the selenoprotein-deficient T-cells to proliferate in response to TCR stimulation was restored by NAC treatment. NAC alone did not induce T-cell proliferation. These observations suggest that selenoproteins are essential for reducing ROS generation in T-cells and that ROS production prevents T-cell activation.

Selenoproteins in macrophages

Selenoprotein mRNA, selenoprotein expression and reactive oxygen species levels

To determine the relative expression levels of selenoproteins mRNA in mouse macrophages, the total RNA was isolated from bone marrow-derived normal macrophages and the expression of mRNA levels transcribed from all 24 mouse selenoprotein genes were analysed by real-time PCR. As shown in Fig. 5(A), relatively high levels of selenoprotein mRNA corresponding to GPx1, GPx4, TR1, Sep15, and selenoproteins P, R, K and T and selenophosphate synthetase 2 were expressed. Interestingly, the most highly expressed selenoprotein mRNA in macrophage, like that in T-cells (see earlier), was GPx1 and a major role of this selenoenzyme is as an antioxidant. Similarly, Sep15, GPx4 and selenoproteins P, K and T were also highly expressed in macrophages.

We deleted Trsp in macrophage ($\Delta Trsp^M$) and examined the effects of Trsp loss on selenoprotein expression and function. Initially, we demonstrated that Trsp was no longer expressed in this cell type (Fig. 5(B)). Selenoprotein expression was also examined in macrophages by incubating the bone marrow-derived macrophages from control and $\Delta Trsp^M$ mice in the presence of ^{75}Se and electrophoresing a protein extract on a polyacrylamide gel (Fig. 5(C), left panel). Several selenoproteins whose mRNA were also detected by real-time PCR were visualized on the gel, while the expression of selenoproteins in $\Delta Trsp^M$ cells were almost completely abolished (Fig. 5(C), right panel). The low levels of Sec tRNA and selenoproteins
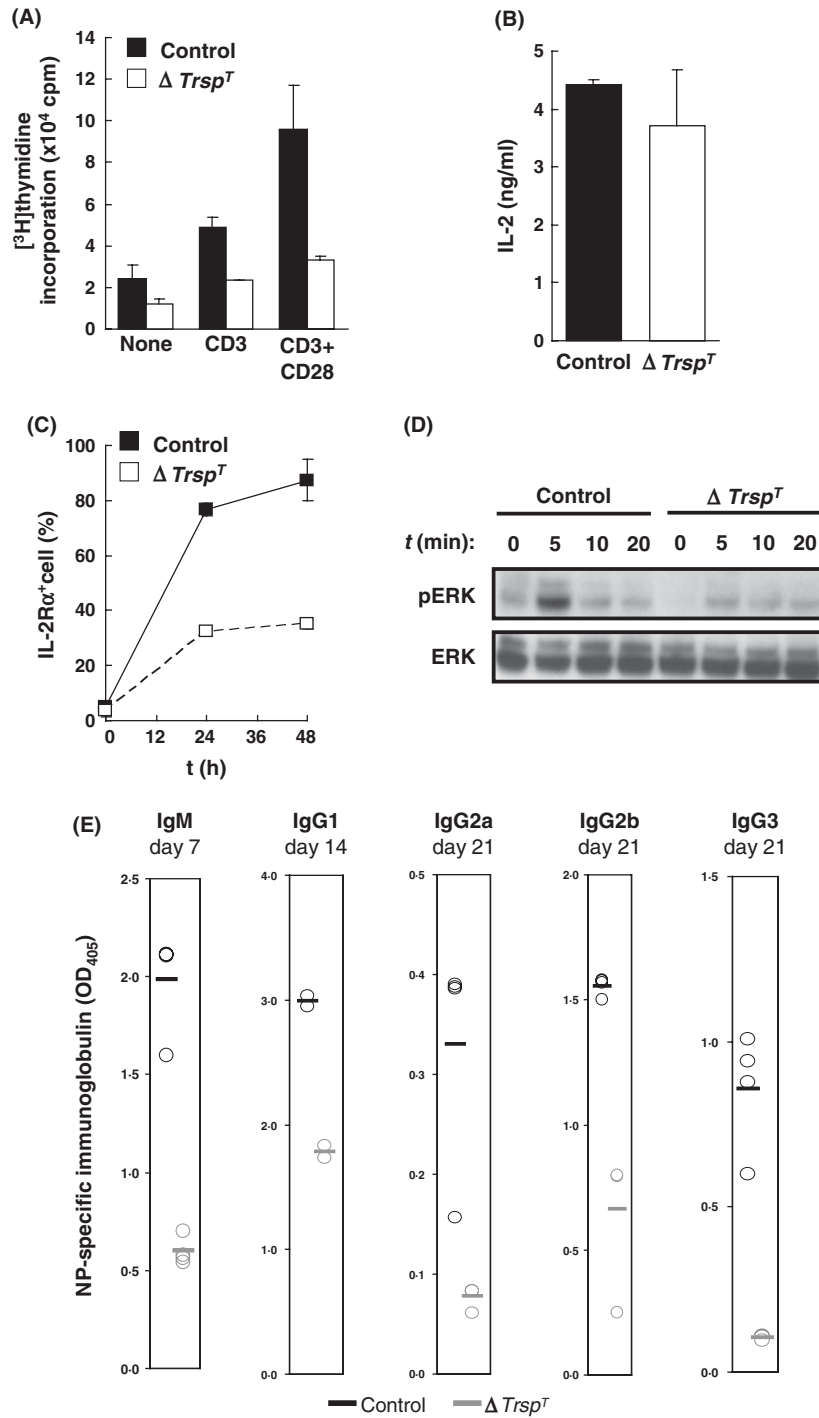
Fig. 3. Activation of control and selenoprotein-deficient-cells ($\Delta Trsp^T$) by T-cell receptor stimulation. T-cells were isolated from lymph nodes of control and $\Delta Trsp^T$ mice. (A) The level of proliferation was assessed by the incorporation of [^{3}H]-thymidine in the absence or presence of anti-CD3/CD28 or anti-CD3 alone. The results of the averages of triplicate determinations from six control and $\Delta Trsp^T$ mice, respectively, are shown. (B and C) The levels of IL-2 production (B) and cell surface expression of IL-2 receptor α (C) in CD3/CD28-stimulated T-cells are shown. (D) Extracellular signal-regulated kinase (ERK) activation in T-cells stimulated with anti-CD3 and anti-CD28 was analysed at the time intervals indicated. Both phosphorylated (pERK) and total (ERK) proteins are shown. (E) Serum levels of major Ig at indicated time points were determined by ELISA in control and $\Delta Trsp^T$ mice following immunization with (4-hydroxy-3-nitrophenyl)acetyl-ovalbumin). Circles represent Ig levels of each animal. Bars represent the mean values. Experimental details are given in Shrimali *et al.*[21].
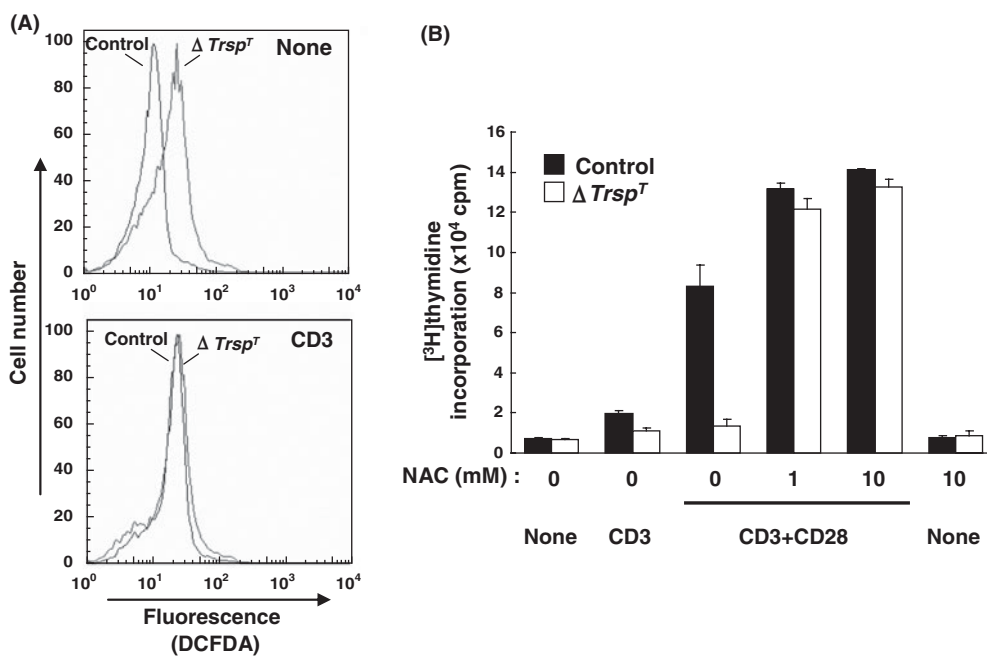
Fig. 4. Reactive oxygen species (ROS) production in T-cell-receptor-stimulated T-cells. (A) Control and $\Delta Trsp^T$ T-cells isolated and purified from lymph nodes and either unstimulated or stimulated with anti-CD3 antibody were used. ROS production was analysed by flow cytometry using 2′-7′-dichlorofluorescin diacetate. (B) Control and $\Delta Trsp^T$ T-cells were either unstimulated (None) or stimulated as indicated in the presence of various concentrations of N-acetyl cysteine (NAC). T-cell proliferation and all other determinations used in the figure are described in Shrimali et al.[21].

observed in $\Delta Trsp^M$ macrophages in Fig. 5(B and C) are likely due to the contamination of the bone marrow-derived macrophage preparations with non-macrophage cells as has been observed in studies involving other cell types[27].

The functions of only about half of selenoproteins have been characterized and most are oxidoreductases and/or antioxidants[31]. Since selenoprotein-less T-cells accumulate higher ROS levels than the corresponding control cells (see Fig. 4(A)), ROS levels in $\Delta Trsp^M$ macrophages were also assessed by staining (Fig. 5(D and E)). Resting selenoprotein-deficient macrophages manifested higher steady state ROS levels than control macrophages.

In vitro and in vivo inflammatory responses

Due to the highly significant role that macrophages have in initiating inflammation, it was important to assess the ability of the corresponding selenoprotein-deficient T-cells in the inflammation process. Macrophages generate cytokines in response to tissue injury and microbial infection. The fact that several inflammatory stimuli such as lipopolysaccharide (LPS) cause transient ROS accumulation as part of cellular signalling events[32,33], and several selenoproteins, e.g. GPx isoforms, which are known to regulate inflammatory responses[34,35], prompted us to examine the effect of selenoprotein deficiency and the resulting redox imbalance on macrophage inflammatory response. We initially treated control and $\Delta Trsp^M$ macrophages with LPS and examined the degradation and replenishment of IκBα. It should be noted that the transcription factor NF-κB, and mitogen-activated protein kinase (ERK, c-Jun N-terminal

kinase and p38) signalling pathways are essential for cellular responses to inflammatory stimuli and that both the degradation and replenishment of IκBα are indicative of NF-κB activation. However, in LPS-treated control and $\Delta Trsp^M$ macrophages, the degradation and replenishment of IκBα occurred independently of the selenoprotein status and the induction of the active forms of the protein kinases (i.e., phosphorylated ERK, JNK and p38) was similar and therefore considered normal[22]. Furthermore, comparison of LPS-induced inflammatory gene expression in the two bone marrow-derived macrophage groups and analyses of gene expression by real-time PCR revealed that the magnitude and kinetics of expression of the genes encoding the chemokines KC (Cxcl1), macrophage inflammatory protein-2 (Cxcl2) and the cytokines TNF-α and IL-1β were similar[22].

We next turned our attention to examining the effects of selenoprotein deficiency in macrophages on the pathology of mice by exposing control and $\Delta Trsp^M$ mice to various models of inflammatory response. The models used included zymosan-induced peritonitis, LPS endotoxemia and chemical irritant (12-O-tetradecanoylphorbol-13-acetate) dermatitis. In these models, control and $\Delta Trsp^M$ mice had comparable rates of neutrophil infiltration, rates of mortality and levels of cytokine production and local edema formation. Since in vitro and in vivo inflammatory responses in both control and $\Delta Trsp^M$ macrophages were similar, we have not shown the data, but refer the reader to the original work[22]. Interestingly, the loss of selenoprotein expression and the deregulated ROS generation in $\Delta Trsp^M$ macrophages did not appear to cause any detectable detrimental effects concerning inflammatory responses.
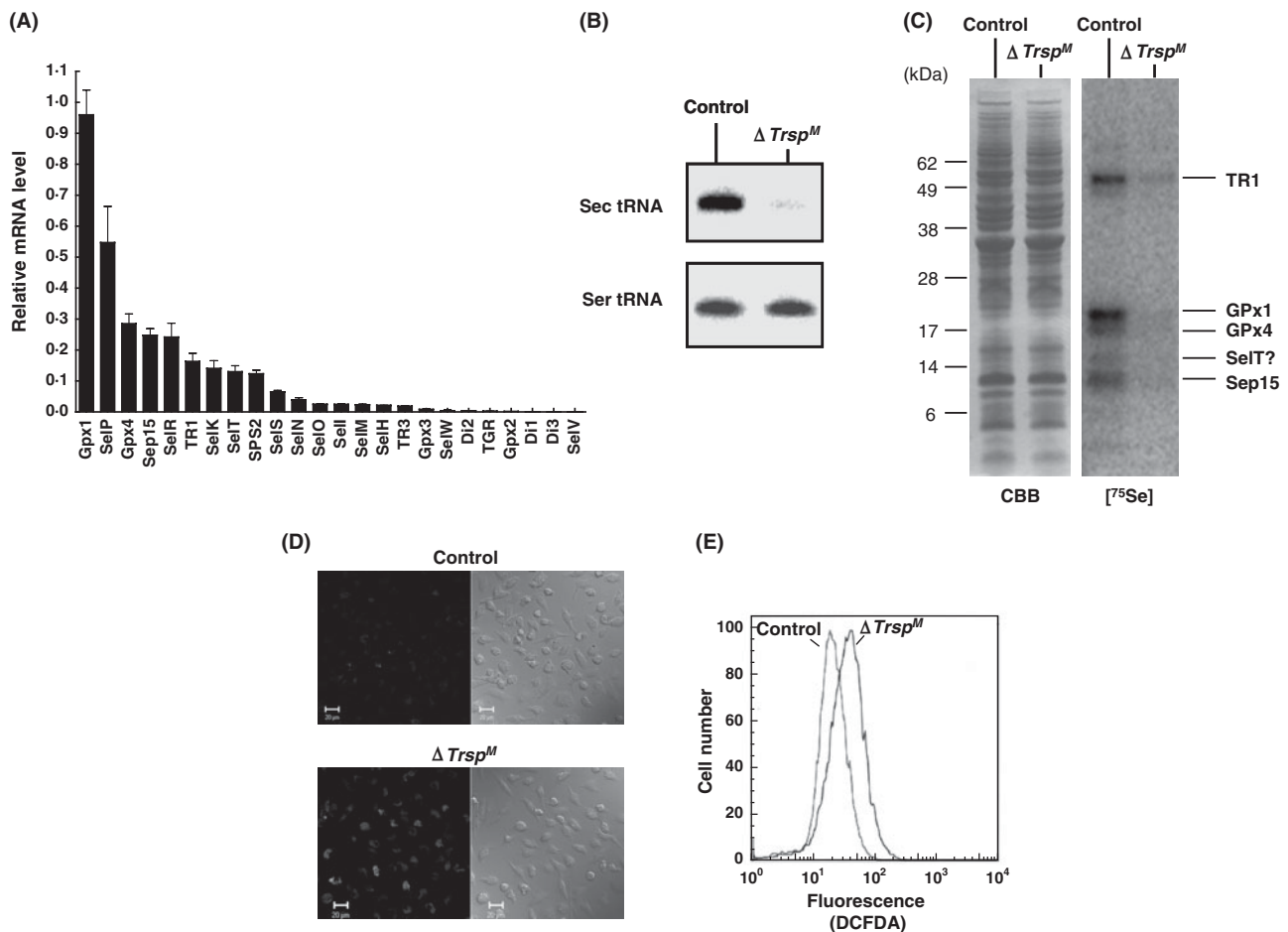
Fig. 5. Selenoprotein gene expression in macrophages. (A) Selenoprotein gene expression in macrophages was analysed by real-time PCR and is shown as the relative mRNA level to that of Gusb which was used as the internal control. (B) Northern blot analysis showing levels of Sec tRNA in control and Δ*Trsp* macrophage. (C) ^{75}Se-labelled selenoproteins were visualized by autoradiography after SDS electrophoresis in control and Δ*Trsp* macrophage as shown in the right panel. The left panel shows Coomassie Blue-stained (CBB) gel, which served as a loading control. The identities of the major labelled selenoproteins are designated on the right of the panel. (D) Macrophages were stained with 2′-7′-dichlorofluorescin diacetate (DCFDA) and reactive oxygen species (ROS) production analysed by confocal microscopy. Fluorescence (left panels) and phase contrast (right panels) images are shown. (E) Macrophages were stained with DCFDA and ROS production analysed by flow cytometry. Experimental details of the studies shown in this figure are given in Carlson *et al.*[22].
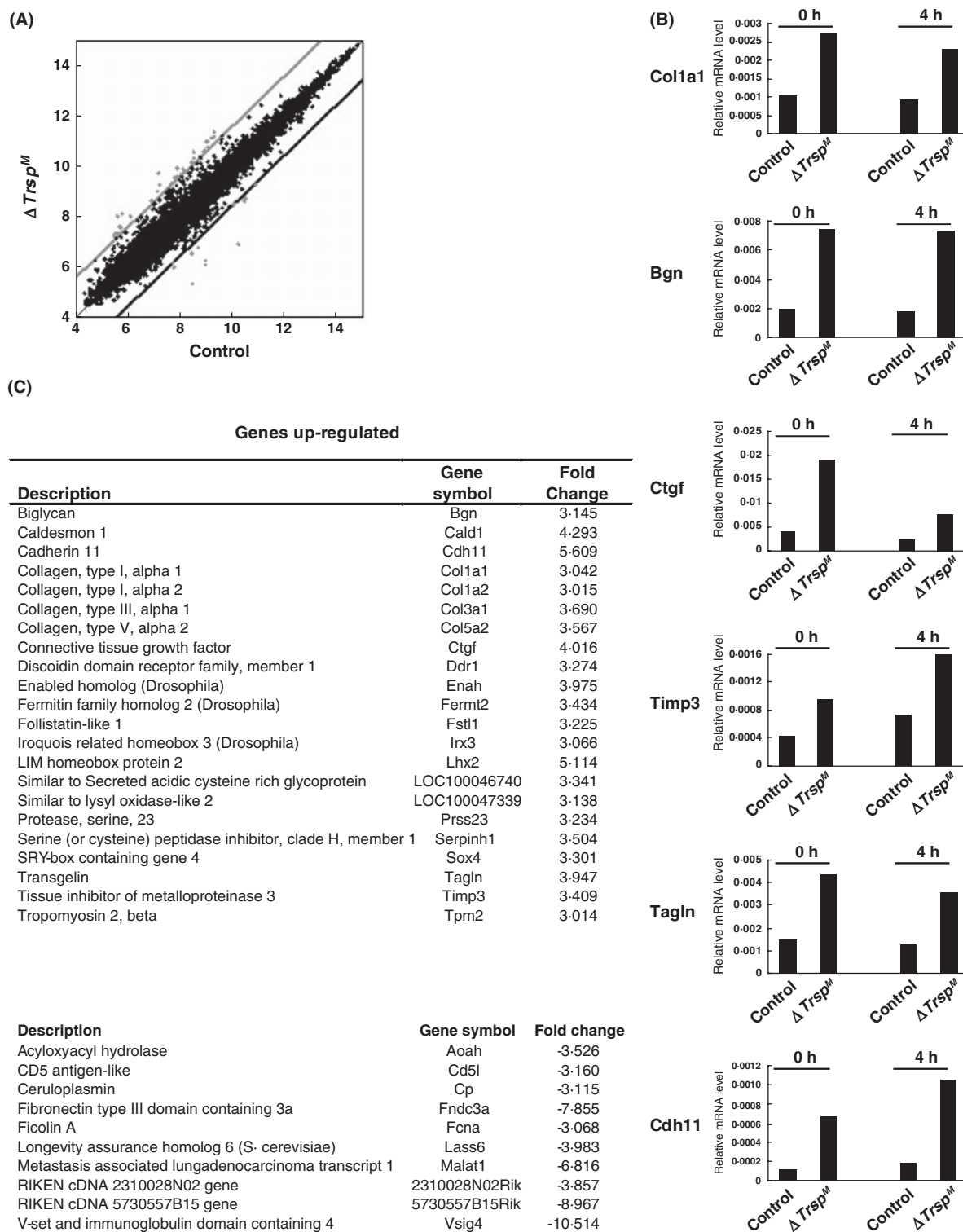
Macrophage microarray analysis

Since there were unexpectedly little or no distinguishable inflammatory phenotypes in Δ*Trsp*^{M} mice, it seemed reasonable that selenoproteins may have other functions in macrophages in addition to roles in inflammatory gene expression. To explore this possibility, an analysis of the global gene expression profiles was carried out in control and Δ*Trsp*^{M} macrophages. We used whole-genome DNA microarrays from mice to assess the expression levels of mRNA in the two macrophage groups. Those genes that manifested a significantly higher or lower expression in Δ*Trsp*^{M} macrophages compared to control macrophages are shown in Fig. 6(A) and are designated by those dots (designating mRNA) that fell outside the lines above and below normal expression levels indicating significant changes.

The expression levels of those mRNA that showed significant changes in selenoprotein-deficient macrophages were verified by real-time PCR analysis (Fig. 6(B)). Interestingly, many genes that deviated significantly in their expression levels from normal in Δ*Trsp*^{M} macrophages were functionally related to the formation, remodelling cellular interaction with the extracellular matrix (ECM). Each of the ECM-related genes that we found to be significantly altered in Δ*Trsp*^{M} macrophages were up-regulated compared to the corresponding control cells (Fig. 6(C)). These included genes that encode ECM components (collagen chains, extracellular proteoglycans and secreted glycoproteins; *Col1a1*, *Col5a2*, *Bgn* and *Ctgf*), inhibitors of ECM proteolysis (metalloendopeptidase and serine-type endopeptidase inhibitors; *Timp3* and *Serpinh1*) and ECM-induced cytoskeletal remodelling (actin-binding proteins; *Tagln*, *Enah* and *Cald1*).
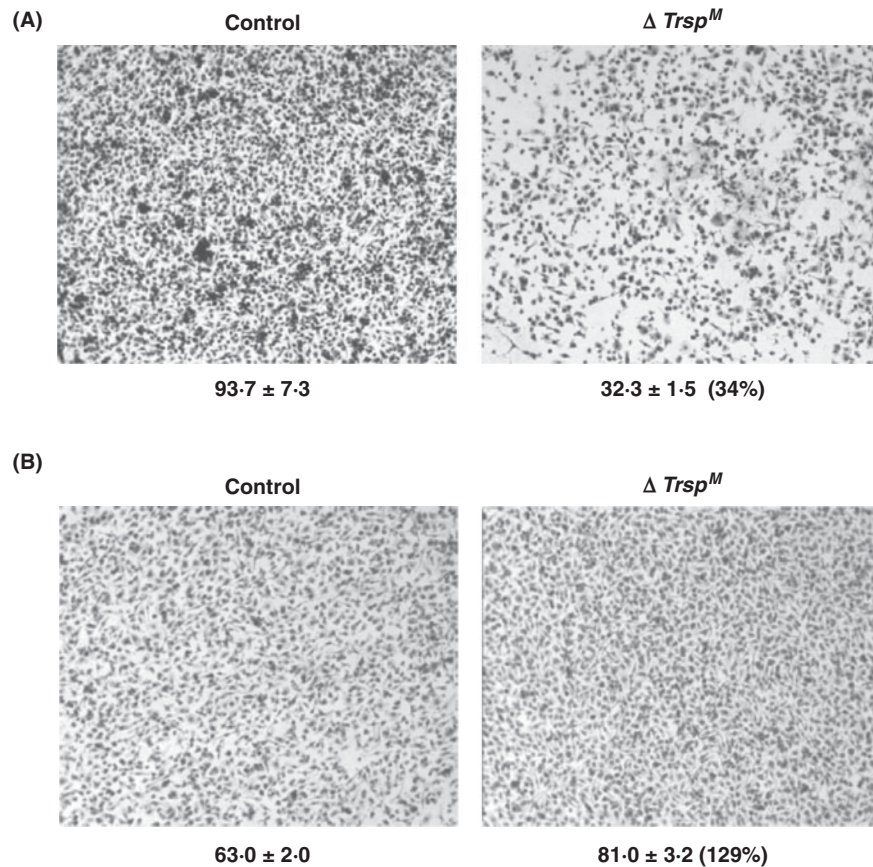
Selenoprotein deficiency and macrophage invasiveness

The microarray analysis of Δ*Trsp*^{M} macrophages suggested that macrophage–ECM interactions were altered by the lack of selenoprotein expression in such a manner that

(A)

(B)

(C)

Genes up-regulated

Description	Gene symbol	Fold Change
Biglycan	Bgn	3·145
Caldesmon 1	Cald1	4·293
Cadherin 11	Cdh11	5·609
Collagen, type I, alpha 1	Col1a1	3·042
Collagen, type I, alpha 2	Col1a2	3·015
Collagen, type III, alpha 1	Col3a1	3·690
Collagen, type V, alpha 2	Col5a2	3·567
Connective tissue growth factor	Ctgf	4·016
Discoidin domain receptor family, member 1	Ddr1	3·274
Enabled homolog (Drosophila)	Enah	3·975
Fermitin family homolog 2 (Drosophila)	Fermt2	3·434
Follistatin-like 1	Fstl1	3·225
Iroquois related homeobox 3 (Drosophila)	Irx3	3·066
LIM homeobox protein 2	Lhx2	5·114
Similar to Secreted acidic cysteine rich glycoprotein	LOC100046740	3·341
Similar to lysyl oxidase-like 2	LOC100047339	3·138
Protease, serine, 23	Prss23	3·234
Serine (or cysteine) peptidase inhibitor, clade H, member 1	Serpinh1	3·504
SRY-box containing gene 4	Sox4	3·301
Transgelin	Tagln	3·947
Tissue inhibitor of metalloproteinase 3	Timp3	3·409
Tropomyosin 2, beta	Tpm2	3·014

Description	Gene symbol	Fold change
Acyloxyacyl hydrolase	Aoah	-3·526
CD5 antigen-like	Cd5l	-3·160
Ceruloplasmin	Cp	-3·115
Fibronectin type III domain containing 3a	Fndc3a	-7·855
Ficolin A	Fcna	-3·068
Longevity assurance homolog 6 (S· cerevisiae)	Lass6	-3·983
Metastasis associated lungadenocarcinoma transcript 1	Malat1	-6·816
RIKEN cDNA 2310028N02 gene	2310028N02Rik	-3·857
RIKEN cDNA 5730557B15 gene	5730557B15Rik	-8·967
V-set and immunoglobulin domain containing 4	Vsig4	-10·514

Fig. 6. Microarray analysis of $\Delta Trsp^M$ macrophages. (A) Genes that were up-regulated and down-regulated in $\Delta Trsp^M$ macrophages are shown in the scatter plot. The ordinate and abscissa represent the logarithmic values of the signal intensity of individual microarray spots. The upper and lower cut-off lines illustrate the margins of gene expression ratio wherein 3-fold or higher up-regulated genes (spots above the light grey line) and 3-fold or lower down-regulated genes (spots below the dark black line) are shown as outside the upper and lower cut-off lines, respectively. (B) Those genes whose expression levels were identified in DNA microarray analysis manifesting an alteration greater than 3-fold were validated by real-time PCR. The relative expression of genes in macrophages that were either untreated or treated with lipopolysaccharide is shown. (C) Expression of genes and protein products that were up-regulated by selenoproteins is shown. Experimental details of the studies shown in this figure are given in Carlson et al.[22].

(A)

Control	Δ *Trsp*^{M}
93·7 ± 7·3	32·3 ± 1·5 (34%)

(B)

Control	Δ *Trsp*^{M}
63·0 ± 2·0	81·0 ± 3·2 (129%)

Fig. 7. Analysis of Δ*Trsp*^{M} macrophage invasion in a protein gel matrix. (A) Macrophage migration was examined by a transwell assay with a protein gel matrix (Matrigel) layer in the upper chamber. Numbers below each panel indicate the relative migration of macrophages and they represent the mean and standard deviation (*n* 3). Numbers in parenthesis indicate the percentage of cell migration in the Δ*Trsp*^{M} sample relative to the control sample. (B) Macrophage migration was examined by a transwell assay in the absence of a Matrigel layer and the data are presented as in (A). Experimental details of the studies shown in this figure are given in Carlson *et al*.[22].

matrix remodelling was decreased and the surrounding ECM was reinforced. Such effects might be expected to impair the migration of macrophages through the ECM and basement membrane. To test this possibility, we compared the invasiveness of control and Δ*Trsp*^{M} macrophages in a protein gel matrix. The migration of selenoprotein-deficient macrophages was dramatically reduced in the gel-laden transwell chambers (Fig. 7(A)). We repeated this assay, but without the gel in the transwell chamber to assess whether the gel invasion phenotype arose from a cell-intrinsic motility defect. Surprisingly, the migration of Δ*Trsp*^{M} macrophages was slightly higher than control macrophages (Fig. 7(B)) suggesting that the changes observed in ECM-related gene expression in macrophages appeared to decrease the ability of the cell to migrate only in surroundings consisting of ECM components.

Conclusion

Our data show that selenoproteins have an essential role in T-cell proliferation in response to TCR stimulation.

Although there is substantial evidence that many selenoproteins function as antioxidants[31], the fact that NAC can compensate for the loss of selenoprotein expression in T-cells and reverse their defect of TCR-induced proliferation provides further evidence that members of this Se-containing protein class serve to maintain redox homeostasis. Not unexpectedly, we observed elevated ROS generation in T-cells and macrophages. The fact that the ROS dysregulation in Δ*Trsp*^{M} macrophages did not result in altered inflammatory responses suggested that their increased levels and oxidative damage in selenoprotein-deficient T-cells were below the threshold to elicit effecting inflammatory responses. Selenoproteins were shown to also have a role in macrophage invasiveness.

Since GPx4 is one of the more highly expressed selenoproteins in both T-cells and macrophages, we are currently examining the effect of targeting the removal of this essential selenoenzyme[36] in these two cell types. In addition, we are using mouse models to investigate the effects of deficient, adequate and supplemental levels of Se intake on the immune response mediated by T-cells against

infectious disease resistance involving influenza virus (M Beck, B Carlson, R Shrimali, JM Park and D Hatfield, unpublished results) that do not express selenoproteins or express only essential, housekeeping selenoproteins, but not non-essential, stress-related selenoproteins[37]. This study will also allow us to characterize the critical Se metabolites (i.e. selenoproteins or low-molecular weight selenocompounds) involved in the immune function and examine their roles in resistance to infectious diseases. Recent epidemiological and molecular evidence suggest a strong link between metabolic regulatory mechanisms and immune homeostasis[38,39]. Our study has just begun to reveal how Se and selenoproteins function at the crossroads of metabolism and immunity. Future studies will seek to offer deeper insights into the effects of dietary Se on the onset and progression of immune disorders and help devise better-informed public health policies.

Acknowledgements

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