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Toll-like Receptors in InflammAging at Ocular Surface.

Running title: InflammAging and Toll like receptors

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Abstract

Purpose:

To evaluate changes in Toll Like Receptors expression at the ocular surface of healthy control volunteers within age.

Methods:

51 volunteers were enrolled in a pilot observational study. Clinical (OSDI questionnaire, Schirmer test and BUT) and Biomolecular (ICAM1, Goblet cells) biomarkers were assessed in all subjects. Temporal Conjunctival imprints were used for molecular analysis while nasal ones were harvested for epifluorescence microscopy.

Results:

Within the age in our sample OSDI score increases, T-BUT values decrease, and goblet cells showed a decreasing trend. Relative real time PCR detected up-regulation of TLR2 and down-regulation of TLR7, TLR8 and MyD88 transcripts. Immunofluorescence data corroborated the PCR results reporting increased TLR2 and decreased TLR7 and TLR8 expression in elder population. A direct correlation was showed between increasing ICAM and increasing TLR2 changes with age.

Conclusion:

Changes in TLR expression are associated with aging, suggesting physiological role of TLRs in modulating innate and adaptive ocular surface immunity. TLRs age related changes may participate to the failure of ocular surface homeostatic mechanisms with inflammAging.

Keywords: TLRs, InflammAging, Para-inflammation, Ageing, Ocular Surface.

Introduction

Aging is a physiological process which leads to several structural and physiological changes. Particularly a dysregulation of the local innate and adaptive immune responses to environmental injuries has been proved.[1] A persistent subclinical low-grade inflammation has been demonstrated at ocular surface of elder self-considering ‘‘healthy’’ volunteers.[2] In those a dysregulation of para-inflammatory mechanisms, which keep tissue homeostasis against environmental injuries or insults, causes an ocular surface system failure, also defined InflammAging. Therefore ocular discomfort due to biomolecular impairments can seriously impact the quality of life of our older patients.[2]

Epithelial cells also respond to stressors by releasing several inflammatory mediators which may influence the local immune state and overall tissue equilibrium.[2] In fact, corneal and conjunctival epithelial cells express numerous immune related recognition receptors, including Toll-Like Receptor (TLR).[3,4] TLRs actively participate in immunological tutoring and innate defense responses at ocular surface.[3] Particularly TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11, which are expressed on cell surfaces, recognize mainly microbial membrane components such as lipids, lipoproteins and proteins, while TLR3, TLR7, TLR8 and TLR9, which are expressed exclusively in intracellular vesicles, such as the endoplasmic reticulum (ER), endosomes, lysosomes and endo-lysosomes, mainly link nucleic acids.[5]

TLRs expression at epithelial and antigen presenting cells (APC) of the ocular surface seems to be relevant in driving innate/adaptive cross-talk of immune cells.[3,4] In fact Although TLRs are essential for protective immunity against infection, inappropriate TLRs responses contribute to acute and chronic inflammation, as well as to systemic autoimmune diseases. More importantly, evidence suggests their critical role in scavenging endogenous molecules produced by dying cells in normal or pathological conditions, usually leading a controlled and self-limited sterile inflammatory response.[5]

It has been also unveiled TLRs role in repairing damaged tissue beyond anti-microbial defense, as in the case of ischemia reperfusion injury.[6] In noninfectious inflammatory diseases such as atherosclerosis and Alzheimer’s disease, oxidized low-density lipoprotein and amyloid, respectively, trigger sterile inflammation.[7] The activation of TLR signaling during tissue damage suggests that endogenous molecules serve as TLR agonists for maintenance of homeostasis, such as tissue repair.[5] Therefore, TLRs may

participate in ocular surface immune homeostasis and their changes within the age might also modify ocular surface para-inflammatory equilibrium causing its age-related dysregulation, hence inflammAging.[8]

Then, our aim in the present study was to evaluate age-related TLRs changes at the ocular surface of "healthy" volunteers, and its impact on tissue inflammAging.

Materials and Methods

Fifty-one consecutive volunteers were enrolled in an open observational pilot study between January 2018 and July 2018. Volunteers were categorized into 3 groups according to age: young (22-40 years), middle-aged (41-60 years) and elder (61-85 years), as per previous study.[2,9] Eligible subjects were at least 18 years of age and described themselves as "healthy". Exclusion criteria included history and/or signs/symptoms of ocular surface disease as well as contact lens use. Further exclusion criteria included systemic conditions (including cardiovascular, metabolic, neoplastic and psychiatric diseases) and pregnancy. In addition, all patients using any topical and/or systemic medications within the three-month period preceding the examination were excluded.

Patient recruitment was carried out at Ophthalmology Complex Operative Unit (University Campus Bio-Medico, Rome, Italy). All study procedures were performed in accordance with guidelines established by the Association for Research in Vision and Ophthalmology, reviewed and approved by the Intramural Ethical Committee(University Campus Bio-Medico, Rome, Italy), observing the tenets declaration of Helsinki. All participants provided a written informed consent to proceed for clinical and laboratory analysis.

Clinical assessment

Ocular surface examination included slit lamp assessment of ocular surface, Tear Break-Up Time (T-BUT), Schirmer Test Type 1, and administration of Ocular Surface Disease Index (OSDI) questionnaire to collect symptoms related to ocular surface irritation and/or discomfort, according to a standard procedure.[10]

OSDI is a 12-item validated questionnaire designed to provide a rapid assessment of symptoms related to ocular surface irritation and their impact on vision-related functioning in patients with dry eye disease (13-22 mild, 23-32 moderate and over 33 severe).

Conjunctival imprints

Two imprints (impression cytology, ICs) from each bulbar conjunctiva (temporal and nasal ones) were sampled using a Millicell filter (0.22μm membrane; Millipore, Milan, Italy). ICs were quickly fixed (citofix, Bio-Fix spray; Bio-Optica, Milan, Italy), according to a standardized procedure.[2] One imprint was used for immunofluorescence and the other was processed for molecular analysis. All imprints were processed for simultaneous RNA and native protein collection, by using the MirvanaParis kit.

Plasticwares, analytical grade chemicals and other minor reagents were purchased from EuroClone (Milan, Italy), Sigma Aldrich (St. Louis, MO, USA), ICN Biomedicals (Milan, Italy) and Carlo Erba (Milan, Italy) or elsewhere described in detail.

Relative real-time PCR: cDNA synthesis and specific amplification

Total RNA was extracted from no-pooled ICs by using the Mirvana Paris extraction procedure (Ambion Ltd., Huntingdon, Cambridgeshire, UK), according to the manufacturers' suggestion with minor modifications. Total RNA was resuspended in 15μL autoclaved Diethyl pyrocarbonate (DEPC)-treated RNase free MilliQ water (Direct Q5, Millipore Corporation, Billerica, MA, USA). To eliminate any DNA contamination, total RNA samples were treated with RNase-free DNaseI, according to the supplier's protocol (2U/μLDNAse; Turbo DNA free kit; Ambion Ltd., Huntingdon, Cambridge-shire, UK). Total RNA samples were checked for A280 analysis (RNA purity >1.8; SPECTROstar Nano; BMG LABTECH GmbH, Allmendgrün, Ortenberg) and verified for no DNA contaminants. Equivalent RNA amounts (500ng) were used as template to generate cDNAs in a one-cycler programmable thermocycler (LifePRO/BIOER; Euroclone, Milan, Italy), according to the GoScript™ Reverse Transcription procedure with minor modifications (Promega, Madison, Wisconsin USA). cDNAs were amplified by using the SYBRgreen Hot start PCR Master Mix (Hydra; Biocell, Rome, Italy) in the presence of specific primer pairs (see Table 1). Both Forward (F) and reverse (R) primers (possibly one intron-spanning) used for amplifications were previously designed according to a standard procedure by using the Primer3 software (freely available) and produced by Eurofins Genomics (10pM; Biotech, Ebersberg, Germany) and validate also in these studies. Real-time PCR amplification was carried out in 48-well microplates (Eco™ Illumina; San Diego, CA) amplification profile reported in Table 1. Samples were amplified in triplicate and single cycle threshold (Ct) values were recorded during linear amplification and

normalized to those of referring genes run in parallel ($nCt = Ct_{target} - Ct_{referring}$), according to a standard procedure (normalized ΔCts ±SD).[11,12] Averages were calculated from replicates and expressed as normalized Ct or as expression ratio of a normalized target with respect to referring gene expression (absolute fold changes), according to the ΔΔCt and 2^-ΔΔCt (REST384–2006 software) analysis.[13] Single melting curves were verified by monitoring the amplification at the end of run. Randomly, PCR products (100-300bps) were tested for single-band amplification in a 2.5% agarose gel. Gels were acquired by computerized UVP BioDoc imaging workstation (UVP, LLC, Upland, CA, UK) in the presence of FluoVis (2mg/ml stock solution; 10µl/small agarose gel) as a nucleic acid intercalant (Smobio).

Immunofluorescence and epifluorescent microscopy

Cytofixed ICs were processed for immunofluorescence according to a standard procedure. A brief blocking/permeabilizing step was carried out for 20 minutes (0.8% bovine serum albumin–0.3% Triton X100 phosphate-buffered saline) before addition of primary antibodies: goat polyclonal anti–TLR2 (sc-8689; 1 µg/ml), rabbit polyclonal anti-TLR3 (sc-10740; 1 µg/ml), rabbit polyclonal anti–TLR4 (sc-10741; 1 µg/ml), goat polyclonal anti–TLR5 antibodies (sc-8695; 1 µg/ml), goat polyclonal anti–TLR7 (sc-13208; 1 µg/ml), or goat polyclonal anti–TLR9 (sc-16247; 1 µg/ml) antibodies, all from Santa Cruz Biotech (Santa Cruz, CA). Specific binding of primary antibody was detected by using cy2-conjugated species specific secondary antibodies (1:300; Jackson ImmunoResearch, West Grove, PA). Nuclear staining was performed with 4', 6-Diamidino-2-phenylindole (DAPI; 5µg/mL solution; Molecular Probes, Invitrogen, Milan, Italy) in PBS supplemented with 20µg/mL RNAse (Molecular Probes), to reduce non-specific signal due to cytoplasm/nucleoli RNA background and to improve the quality of nuclear acquisition. Images were acquired using direct Ni direct Eclipse microscope equipped with the DS-Ri1 digital camera (Nikon) and the NIS Elements Imaging Software (Nikon, Tokyo, Japan). Plan fluor X20/0.50 and X40/0.75 objectives, and plan Apo X60/1.40 objective were used for acquisition (Nikon). Isotype-matched IgG antibodies (Vector Labs. Ltd., Burlingame, CA) were incubated in parallel and used as internal controls (background subtraction).

Statistical analysis

Data values are expressed as the mean ± SD (in the text) and mean ± SEM (in the graphics). The statistical

164 package used was StatView II for PC (Abacus Concepts. Inc., Barkley, CA, USA). Differences between groups
165 were assessed with ANOVA followed by a Tukey-Kramer post hoc analysis. Correlations between two
166 variables were evaluated by Pearson's correlation coefficient analysis. $P \leq 0.05$ was selected as the limit of
167 statistical significance.
168
169 **Results**
170 Clinical assessments revealed OSDI scores (young: 7.65±0.71score; middle age: 12.54±0.71 score; old:
171 17.40±0.85 score; $p<0.05$) significantly increased with ageing and correlated negatively with Goblet cells
172 number ($R=-0.8393$, $p<0.0006$). By contrary, BUT values decreased with ageing (young: 11.50±1.81 sec;
173 middle age: 8.75±1.50 sec; old: 8.17±2.50 sec; $p<0.001$) and positively correlated with Goblet cells number
174 (rho=0.3529, $p>0.05$). Schirmer test decreased with aging (young: 20.07±0.74 mm; middle age: 17.85±1.34
175 mm; old: 14.65 ±0.92 mm, $p<0.05$). Demographics are reported in table 2.
176 TLR-2 immunoreactivity increased significantly with aging (young: 39.96±2.08 IntDen; middle age:
177 62.76±0.03 IntDen; old: 69.44±1.43 IntDen) (Fig 1-A).
178 TLR4 immunoreactivity showed a significant increase at middle age (67.78±9.37 IntDen) (Fig. 1-B) as well
179 as TLR-9 (middle age: 35.98±2.93 IntDen, old: 27.58±1.00 IntDen compared to young 25.31±0.46 IntDen)
180 (Fig. 1-C) and TLR7 (middle age: 53.53±0.02 IntDen, old: 43.14±2.40 IntDenyoung: 34.36±1.88 IntDen) (Fig
181 1-E) but all there decreased in elder population (Fig. 1-B,C,E); TLR5 values were similar in all groups.
182 A decreasing reactivity was observed for TLR3 (young: 73.61±1.28 IntDen, middle age 62.34±5.78 IntDen,
183 old: 56.25±5.45 IntDen) (Fig. 1-C,D)
184 Molecular analysis revealed a significant downregulation of TLR4 (-2.55±0.09 2log-fold changes; $p<0.05$) and
185 a trend of reduction of MyD88 (-2.83±0.05 2log-fold changes). Upregulation of TLR2 (4.65±2.05 2log-fold
186 changes, $p<0.05$) in old population compared to young was also evaluated. TLR7 (-4.76±0.01 2log-fold
187 changes, $p<0.05$) and TLR8 (-4.61±0.01 2log-fold changes, $p<0.05$) transcripts were significantly
188 downregulated in elder population (Fig.2-A, B). Not significant changes were observed for TLR5, TLR3 (Fig.
189 2-A, B). An overall increase of cell surface TLRs and a decrease of intracellular ones with ageing was also
190 detected significantly (Fig 3). A direct correlation was showed between increasing ICAM-1 and increasing
191 TLR2 changes within the age. (Fig. 4)

Discussion

TLRs at ocular surface epithelial cells of healthy volunteers changed with the age. Increased expression of cell-surface TLRs with downregulation of cytoplasmatic TLRs was detected. Increasing TLR2 and reducing TLR3 and 7 expression was strictly correlated with increasing age-related expression of I-CAM 1, then aspecific sterile inflammation.

Clinical (T-BUT, Schirmer Test Type 1, and OSDI) and biomolecular (ICAM1 and goblet Cells) biomarkers unveiled an unperceived inflammAging status in our self-considered healthy volunteers.[2] Such clinical and biomolecular changes were significantly correlated to increased TLR2, and reduced TLR7-8 in elder population, unveiling a role of TLRs in ocular surface homeostasis, and not only in microbial defense.[14]

Such "aging patterns" would cause alteration of both innate and adaptive immunity.[2] Age- related alteration of TLRs local expression may be a preclinical and/or subclinical condition that determine the failure of the para-inflammatory compensatory mechanisms to adequately restore ocular surface homeostasis leading to the ocular surface disease.[5,8,14–18]

The Toll-like receptors (TLRs) are primary components of the innate immune system involved in signal transduction and are expressed by entire "healthy" ocular surface, allowing a prompt innate response to pathogenic strains that might trigger local inflammation.[18] The human ocular surface is colonized by an expansive varied microbial community, as evidenced by previous researches, in equilibrium state.[8,14–22] TLRs are taking part in controlling such homeostatic balance at the ocular surface, bridging the gap between microbial flora and ocular surface.

However, they are part of family of membrane-bound pattern recognition receptors (PRRs) located either on the cell surface or in endosomal compartments. These receptors are known to respond to host-molecules termed damage-associated molecular patterns (DAMPs) that have taken on the appearance of ''non-self.'' Sterile inflammation occurs in response to a growing list of DAMPs ranging from oxidized lipids or lipoproteins, to deposits of protein/lipid aggregates or particulate matter.[23]

The eye normally exhibits ''immune and angiogenic privilege'' to avoid the potential sight destroying consequences of ocular inflammation.[24] Such privilege involves anatomical, cellular, and soluble factors, as well as TLRs, ensuring homeostatic equilibrium.[25] Despite these mechanisms to prevent potentially harmful

ocular inflammation, such a state of immune privilege can break down, resulting in a dysregulated sterile inflammatory status.[26]

This sterile inflammation does not show signs of acute inflammation; rather it appears to be low-grade and chronic. Sterile inflammation occurs in response to a growing list of modified host-derived elements ranging from oxidized lipids or lipoproteins to deposits of protein/lipid aggregates or particulate matter.[23] These stimuli trigger activation of pattern recognition receptors (PRRs) of the innate immune system.[27]

In fact frequently these stimuli are often easily cleared, leading to a self-limiting and controlled homeostatic para-inflammation. But, once such system is dysregulated, the inflammatory response can persist causing over-activation of the immune system and contributing to disease pathogenesis.[2,28]

In fact, as in our data[2], with progressive age, increased oxidative damage occurs in many other tissues, including the retina, and is thought to contribute to the progression of multiple forms of retinal degeneration most notably AMD.[28]

TLR2 has an alert sentinel function and is the main recruitment/phagocytic receptor for innate cells in case of bacterial infection[29], and it is also involved in promoting angiogenesis during wound healing.[30,31]

Excessive ROS can damage lipids through a mechanism known[28] and conceivably induce activation of TLR2 in AMD and in other retinal diseases where ROS play a role in pathology TLR2 inhibition provides striking protection to the retina in response to oxidative stress. We show that oxidative stress activates.[28] TLR2 mediates complement deposition in response to oxidative stress that is pathological in nature, and that blocking TLR2 signaling preserves cells integrity in vivo under conditions of acute oxidative stress.[28]

TLR2-activated mononuclear phagocytes by oxidative damage-induced TLR2-mediated C3/MAC activation.[28]

TLR2 ligand on endothelial cells inducing angiogenesis in a VEGF-dependent manner.[31,32]

Therefore with ageing, as shown in our data the absolute and relative increase of TLR2 is directly related to increasing ICAM expression and inflammation, suggesting a dysregulation in TLRs activity. Such TLRs unbalance may be contributing cause of the altered homeostatic mechanisms occurred with ageing.

Healthy corneal epithelium usually do not express TLR2 at the cell surface, failing to elicit immune response to ligands (TLR silent form) [33], but expresses high levels of TLR9, compared with the average expression of TLR-2, whereas the expression by the underside stroma is at similar levels.[34] Therefore the overexpression of

cytoplasmatic TLRs may create an immunosilent condition to prevent unnecessary inflammatory responses.[33] A relative reduction of cytoplasmatic TLR7/8 and TLR9, in old subjects may trigger the activation of the innate as well as adaptive immune response.[35–37]

In fact, although still controversial and debated, several evidences describe the immunomodulating activity of cytoplasmatic TLRS, such as TLR7, 8, 9 and 3, underlining their role in inducing immunotolerance.[38–42] Therefore, an increased expression of TLR2 and a relative decrease of cytoplasmatic TLRs compared to younger population may expose elder population to inflammAging.

However few limitations characterize this study on healthy volunteers: (1) the small number of subjects enrolled and (2) the unfeasibility to perform a complete analysis of TLR protein expression.

Conclusions

Aging has been associated with several ocular dysfunctions and degenerations such as (1) ocular surface failure which may worsen in in dry eye disease and post-surgery discomfort syndrome, (2) Age related macular degeneration. Both conditions are related to a loss of homeostatic mechanisms, and dysregulated parainflammation leading to a tissue failure and sterile subclinical persistent inflammation. InflammAging at ocular surface may be partially caused by a TLRs expression and signaling unbalance. Age related variations in TLR expression in apparently healthy volunteers may influence local immune surveillance and para-inflammatory homeostatic response. These new insights unveil the complexity of the inflammAging but also the critical role of TLRs in immune surveillance, and in regulating the innate and adaptive immune response, hence preventing autoimmunity, at ocular surface.

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AU contribution declaration:

-Design and conduction of the study (ADZ, AM)

-collection, management, analysis and interpretation of the data (all)

-preparation (ADZ, AM, MDP), reviewing (MC, TM), approval (all) of the manuscript

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Figure legend

Table 1. Primer description.

Genes	*Primer pairs (F/R)*	*Annealing temp. °C*
	Referring genes	
H3	F: 5'-ACT CAT CAC CGT AGC CAG GT-3' R: 5'-CAC CTT GGC TTG AGC ACT C-3'	60°C
18S	F: 5'-GGA GAG GGA GCC TGA GAA C-3' R: 5'-AGG GCC TCG AAA GAG TCC T-3'	58°C
	Target genes	
TLR-1	F: 5'-TTC ACA GTG TCT GGT ACA CGC AT-3' R: 5'-ACC GTG TCT GTT AAG AGA TTA TTG GA-3'	58°C
TLR-2	F: 5'-AGT GAG CGG GAT GCC TAC T-3' R: 5'-GAC TTT ATC GCA GT CTC AGA TTT AC-3'	60°C
TLR-3	F: 5'- GCC GCC AAC TTC ACA AGG TAT A-3' R: 5'- AGC TCA TTG TGC TGG AGG TT -3'	58°C
TLR-4	F: 5'-AAT CCC CTG AGG CAT TTA GG-3' R: 5'-CAG GGC TAA ACT CTG GAT GG -3'	58°C
TLR-5	F: 5'-CCA TAG ATT TTT CCT CCA ACC AAA TA-3' R: 5'-TCA TAC ATT TTC CCC AGT CCA CT-3'	58°C
TLR-6	F: 5'-CAT CCT ATT GTG AGT TTC AGG CAT-3' R: 5'-GCT TCA TAG CAC TCA AC CCA AG-3'	59°C
TLR-7	F: 5'-GGA GGT ATT CCC ACG AAC ACC-3' R: 5'-TGA CCC CAG TGG AAT AGG TAC AC-3'	60°C
TLR-8	F: 5'- AAA CTT GAC CCA ACT TCG ATA CCT AA-3' R: 5'-GAT CCA GCA CCT TCA GAT GAG G-3'	60°C
TLR-9	F: 5'-TTC ATG GAC GGC AAC TGT TA-3' R: 5'-GAG TGA CAG GTG GGT GAG GT-3'	58°C
TLR-10	F: 5'-AAG AAA GGT TCC CGC AGA CTT-3' R: 5'-TGT TAT GGC ATA GAA TCA AAA CTC TCA-3'	58°C

Abbreviations: TLR, Toll-like Receptor; H3, Histon 3; 18S, 18S ribosomal RNA
Details in primer design/synthesis and amplification set are shown in the text (M&M).

Table 2. Demographics.

Groups	*N°*	*Age*	*Age ± SD*	*Sex*
Population	51	22-85	49.57 ± 3.76	27F/24M
Young	18	22-40	25.32 ± 1.17	10F/8M
Middle-aged	16	41-60	49.87 ± 5.31	6F/10M
Old	17	61-85	73.47 ± 8.68	11F/6M

Figure 1. immunoreactivity in conjunctival imprints

Epifluorescence acquisitions (overlays; x200) of TLR immunolabeled (green/cy2) imprints over a blue nuclear counterstaining (dapi/blue). Representative merge panels showing the specific TLR2 (A), TLR4 (B) and TLR9 (C) TLR3 (D), TLR7 (E) immunoreactivity, respectively in young (upper), middle age (middle) and old (lower) groups. As shown, an increase of TLR2 was observed by aging. A significant increase of TLR4 was observed in middle age imprints. Histograms are IntDen values according to the ImageJ analysis, after channel split and background normalization. Red line indicates the young expression and * pointed at $p<0.05$ significant level.

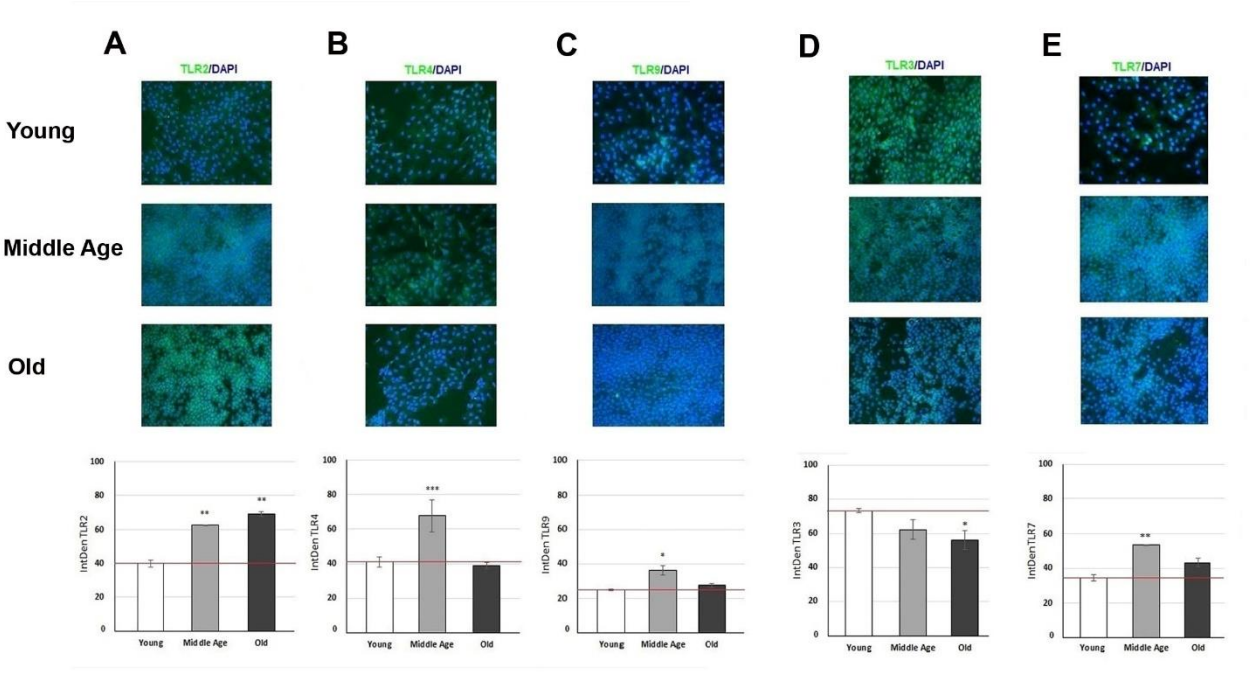
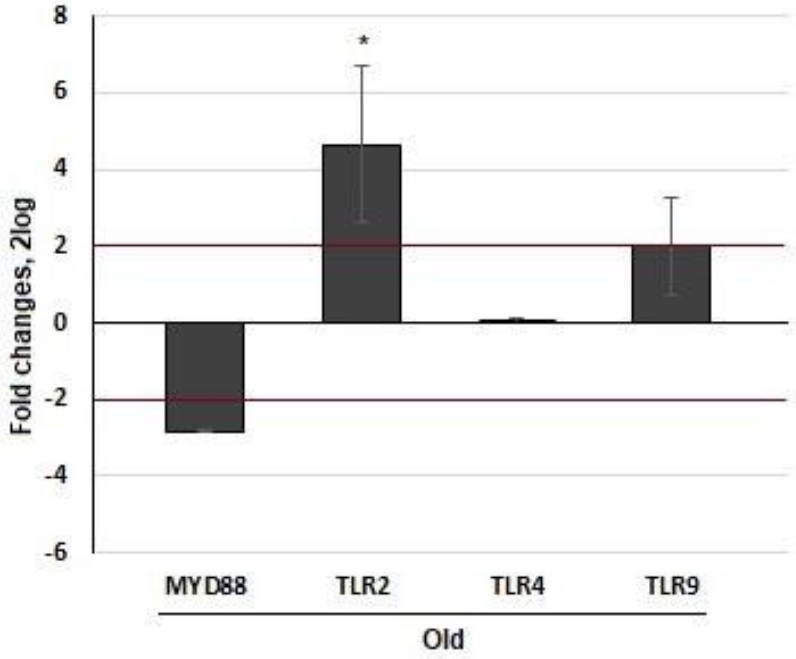


Figure 2 Changes in TLRs transcript expression at aging.

Total RNA was extracted by conjunctival imprints and cDNA was synthetized for specific amplification in relative real time platform. All comparisons were performed against young imprints (REST analysis followed by Tukey-Kramer post hoc. (A) A significant upregulation of TLR2 was observed in old imprints, while no significant changes were detected for TLR4 and TLR9. (B) In old imprints, a significant deregulation was observed for TLR7 and TLR8, while TLR5 was unchanged. Asterisks (*) in histograms point at significant differences between subgroups ($p<0.05$) Dashed line indicate range significance for REST analysis (2log comparisons).

A


8
6
4
2
0
-2
-4
-6
Fold changes, 2log
*
MYD88
TLR2
TLR4
TLR9
Old

B

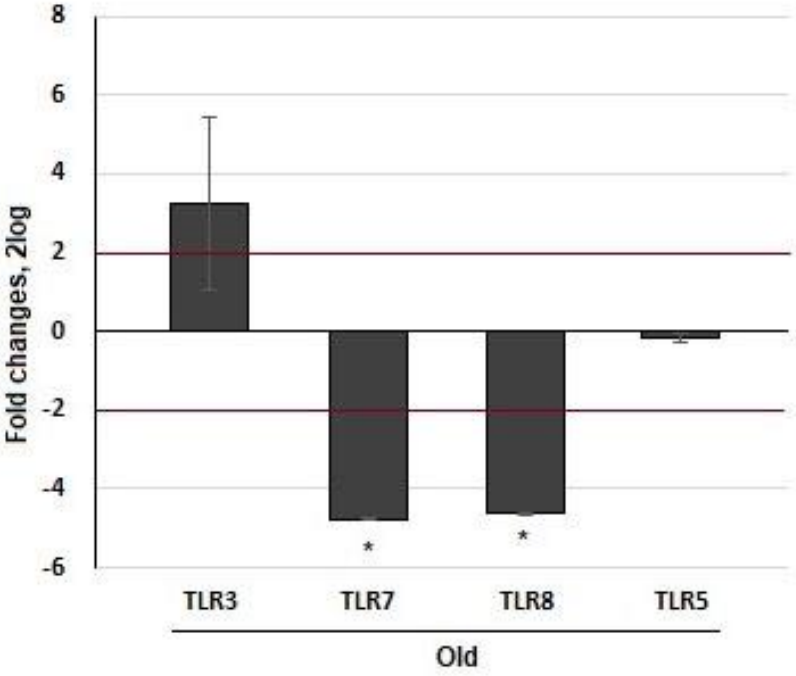

8
6
4
2
0
-2
-4
-6
Fold changes, 2log
*
*
TLR3
TLR7
TLR8
TLR5
Old

Figure 3. Age-related TLRs changes

(A) Epifluorescence acquisitions (overlays; x200) of TLR immune-labeled (green/cy2) imprints over a blue nuclear counterstaining (dapi/blue). (B) Correlation showing significant increasing expression of surface TLRs with aging associated to a (C) reduction of cytoplasmic TLRs. Pearson post hoc: R and p values are reported in the panels

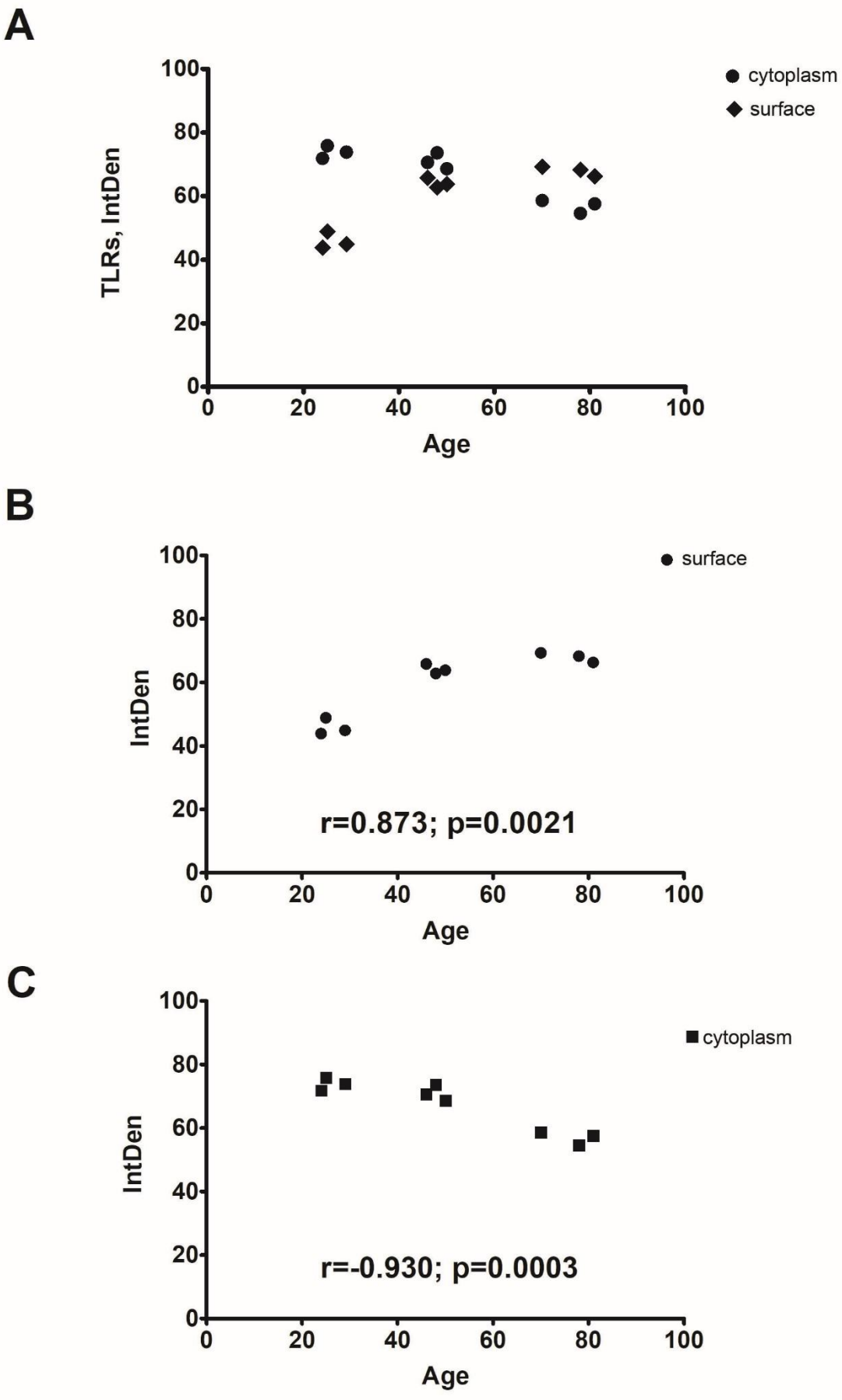
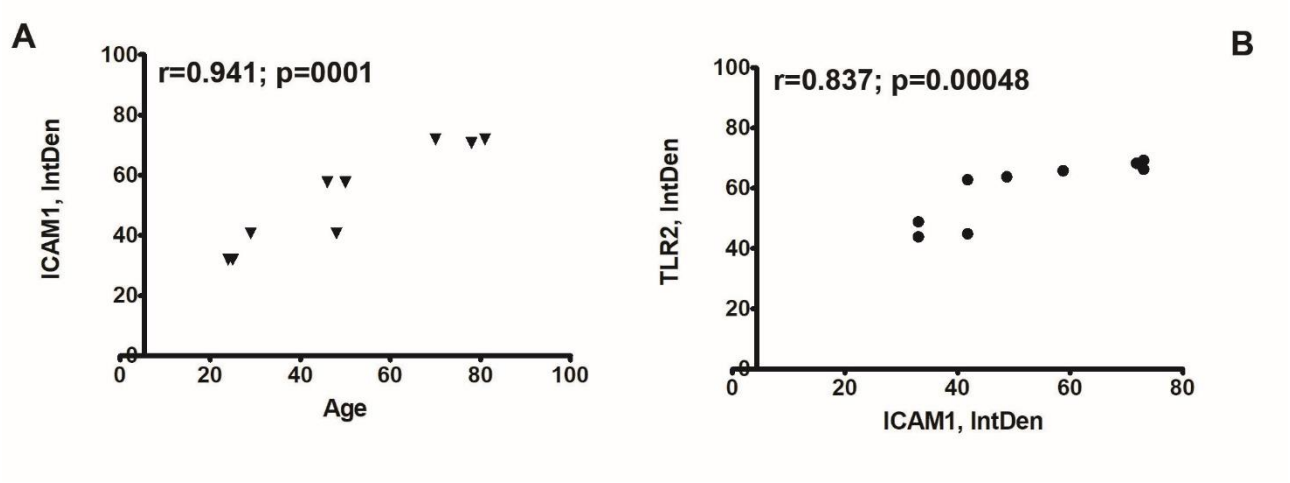

A
cytoplasm
surface
TLRs, IntDen
Age
B
surface
IntDen
r=0.873; p=0.0021
Age
C
cytoplasm
IntDen
r=-0.930; p=0.0003
Age

Figure 4. TLR2 and InflammAging.

(A) I-CAM 1 expression increases with aging, showing biomolecular inflammAging changes in our population. (B) Increasing I-CAM 1 directly correlate with increase TLR2 expression within the age.



Figures

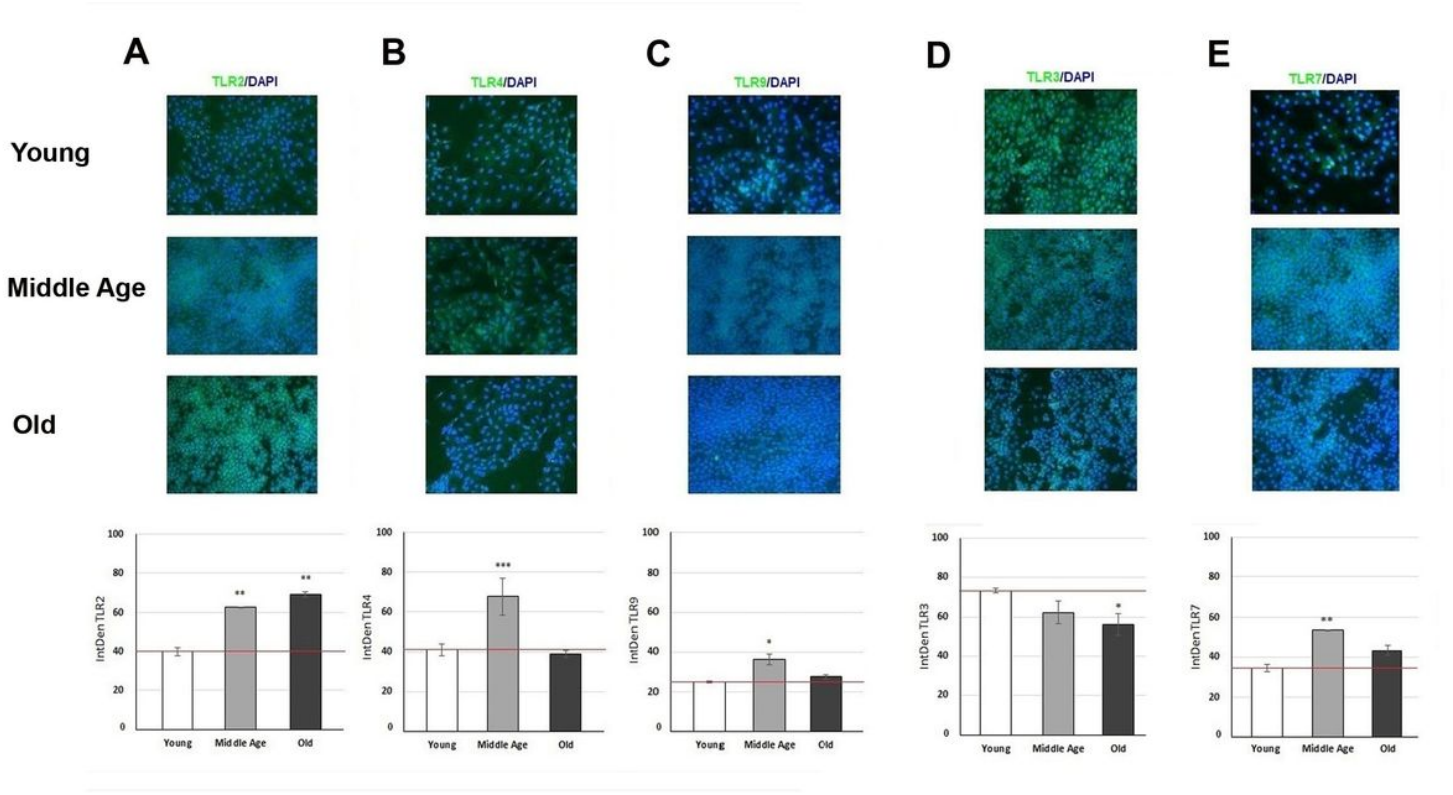


Figure 1

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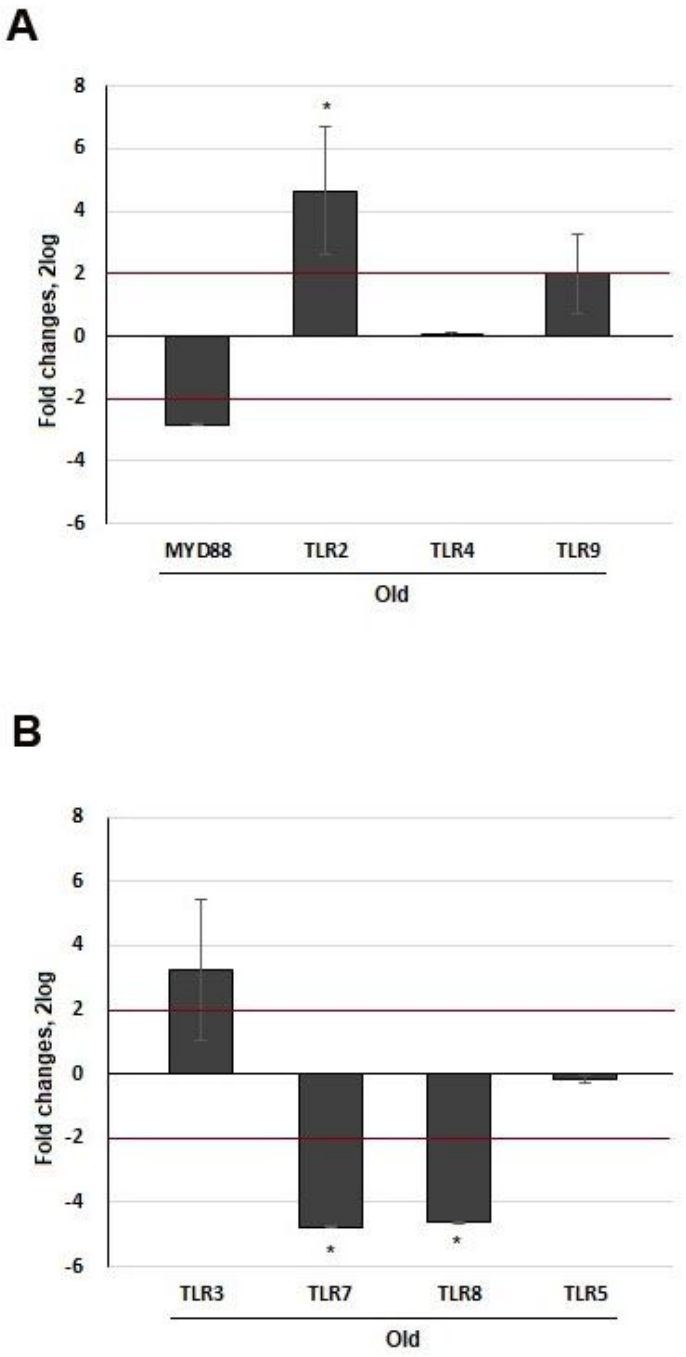

Figure 2

Changes in TLRs transcript expression at aging. Total RNA was extracted by conjunctival imprints and cDNA was synthetized for specific amplification in relative real time platform. All comparisons were performed against young imprints (REST analysis followed by Tukey-Kramer post hoc. (A) A significant upregulation of TLR2 was observed in old imprints, while no significant changes were detected for TLR4 and TLR9. (B) In old imprints, a significant deregulation was observed for TLR7 and TLR8, while TLR5

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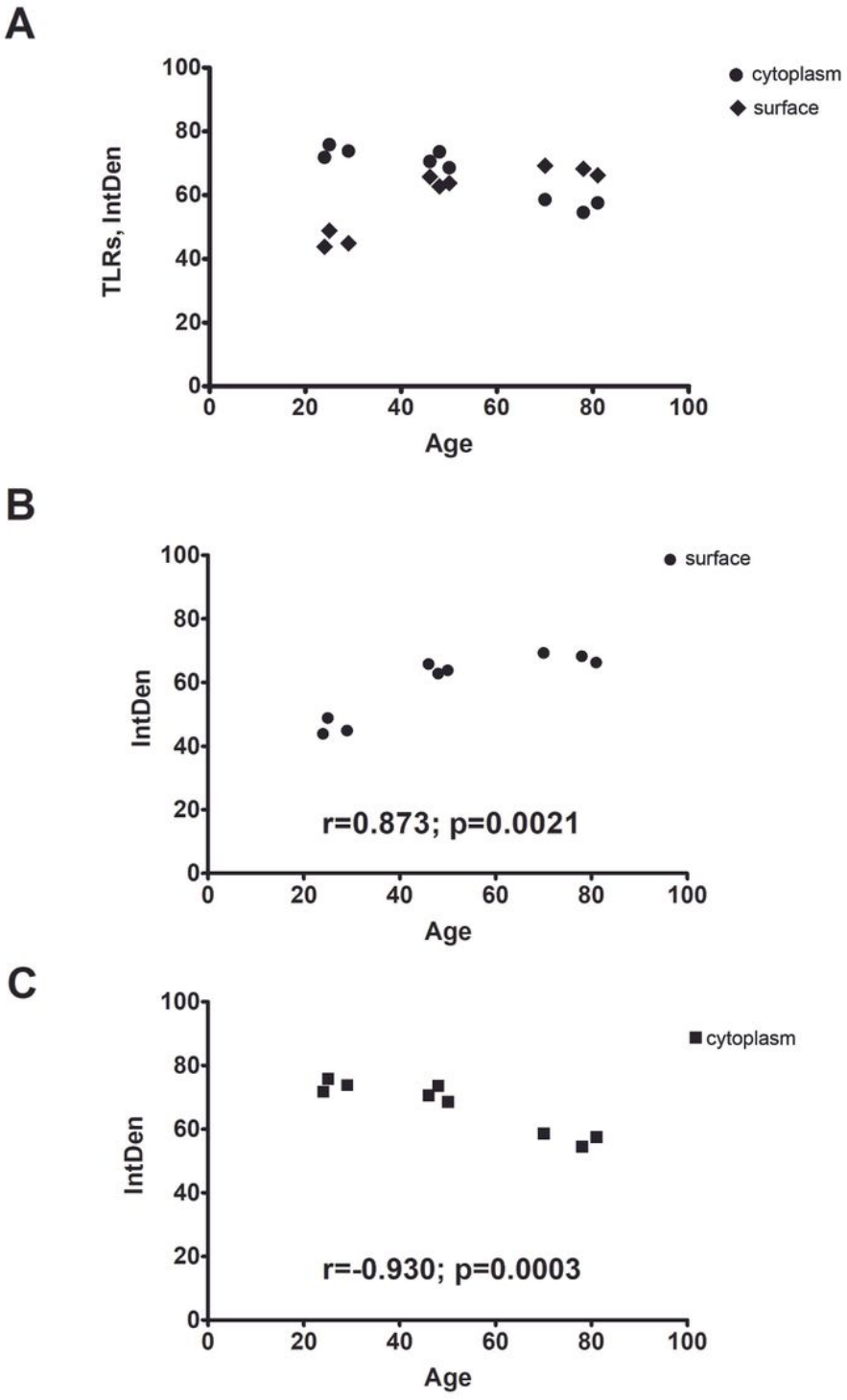

Figure 3

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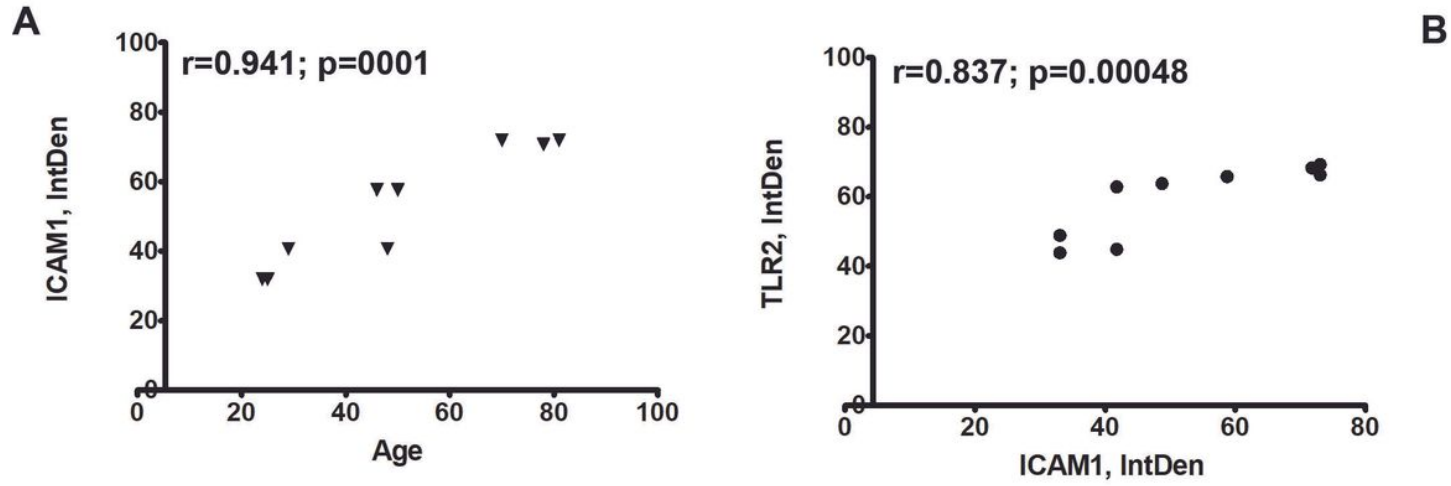

Figure 4

TLR2 and InflammAging. (A) I-CAM 1 expression increases with aging, showing biomolecular inflammAging changes in our population. (B) Increasing I-CAM 1 directly correlate with increase TLR2 expression within the age.