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MOLECULAR FUNDAMENTALS OF INFLAMMATION

Medical University of Graz

PUVA diminishes imiquimod-induced psoriatic phenotype with gene expression signature associated with senescence

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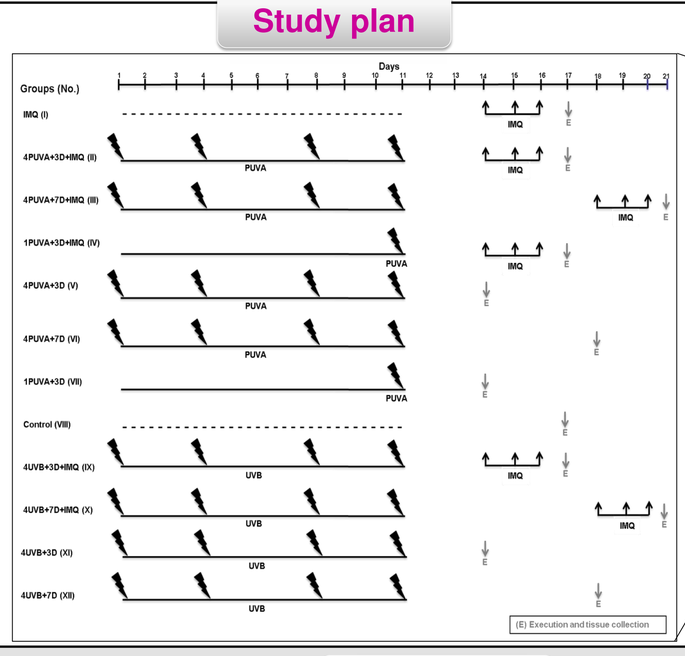
Background

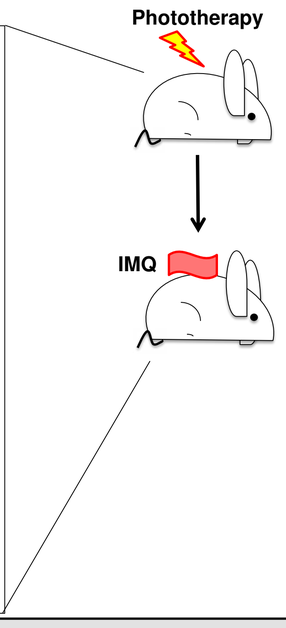
- Psoriasis is a chronic disease for which phototherapy with UVB and psoralen plus ultraviolet A (PUVA) have high clearance rates
- However, in subset of patients PUVA is superior in efficacy and in overall reveals a longer remission rate compared to UVB
- But, the therapeutic mechanisms of PUVA as well as UVB are not yet well understood

Hyothesis

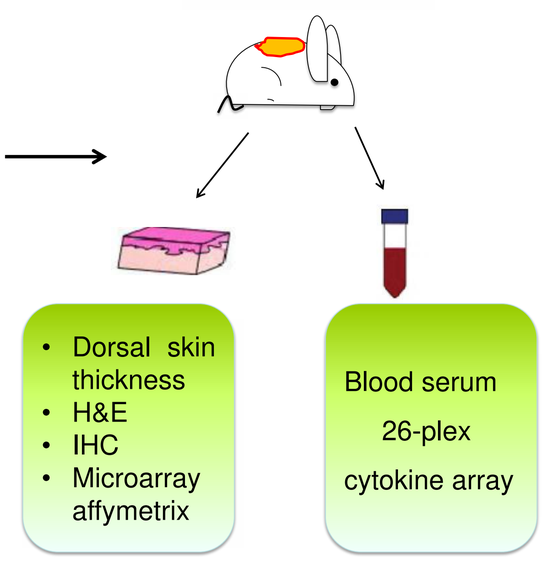
To study the effects of phototherapy pretreatment using sub-phototoxic doses of PUVA (0.25 J/cm^2) or UVB (0.20 J/cm^2) as shown in the study plan, on the psoriatic phenotype development due to 3 applications of IMQ.

Study plan





Methodology



- Dorsal skin thickness
- H&E
- IHC
- Microarray affymetrix

Blood serum
26-plex
cytokine array

Results

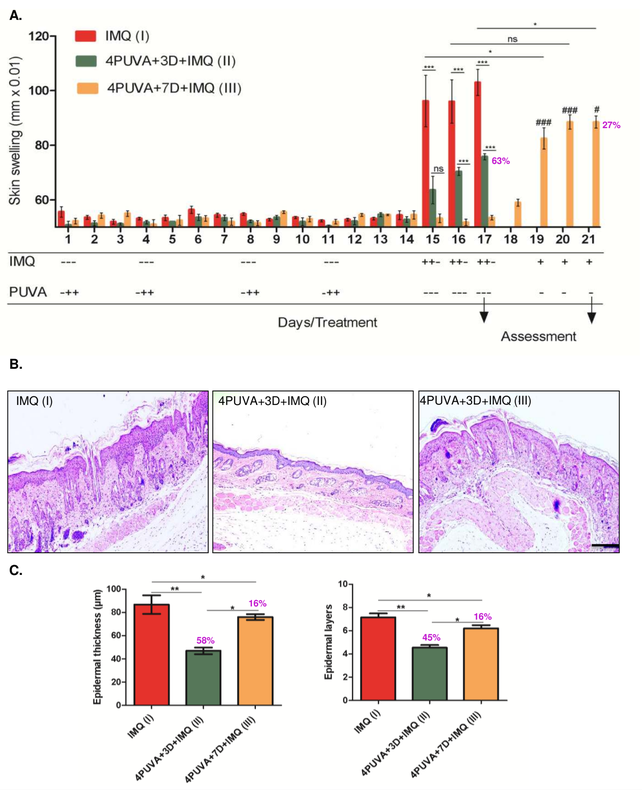
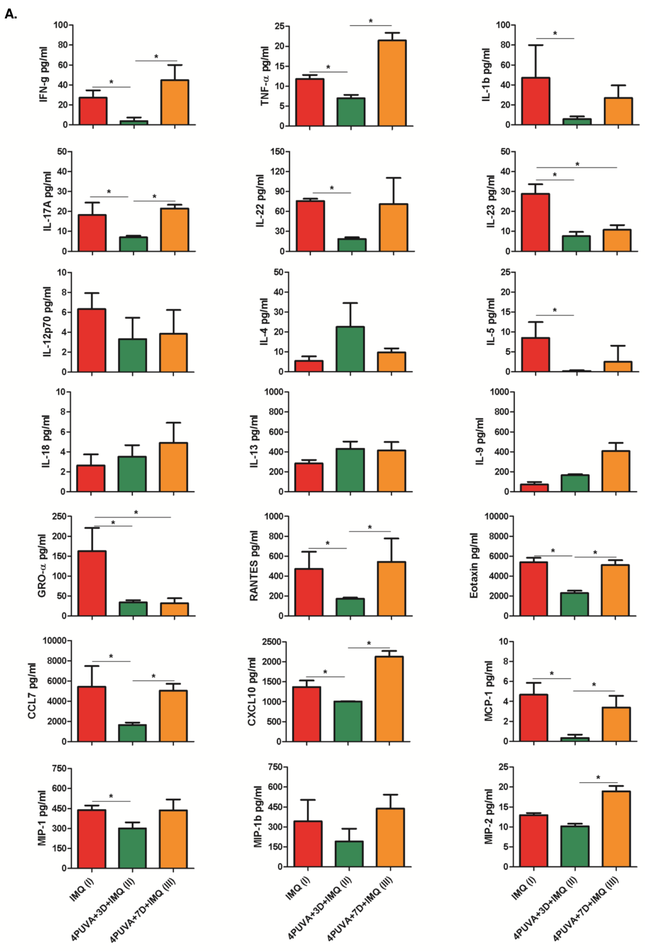


Figure 1. Effect of phototherapy pretreatment on IMQ-induced inflammation. Double skin fold thickness (DSFT) measurement to quantify the change in IMQ-induced thickening of the skin after PUVA (**A**) and UVB (**D**) pretreatment along with their respective suppressive effect in % in comparison to IMQ. Representative image of H&E-stained section from dorsal skin of a mouse pretreated with PUVA (**B**) and UVB (**E**) (scale bar, 200 µm) along with evaluation of histological features such as epidermal thickness (µM) and epidermal layers (**C&F**) depicted with % suppression at the top. Data shown are from one representative experiment, with n=5 mice. Statistical differences between the groups for both studies were determined by Two-way ANOVA using Bonferroni post-test. For PUVA, *p <0.05; **p ≤ 0.01; ***p ≤ 0.001 for Group I vs Group II or Group III and # p < 0.05; ### p ≤ 0.001 for Group III vs Group II. While for UVB, *p < 0.05; ***p ≤ 0.001 and # p < 0.05 for Group I vs Group IX or Group X; ## p ≤ 0.01 for Group X vs Group IX.



B.

Cytokines/chemokines	Serum levels in pg/ml		p-Value
	Control (VIII)	4PUVA+3D (V)	
IFN-gamma	8.9 ± 1.1	1.2 ± 4.2	0.03
IL-17A	26.2 ± 14	0 ± 1	0.05
IL-9	63.3 ± 18	0 ± 0	0.05
GRO-alpha	12.3 ± 10.7	34 ± 9.6	0.05
Eotaxin	3620 ± 1219	6067 ± 1373	0.11
IL-23	0.17 ± 0.29	0.83 ± 0.76	0.13
IL-13	13.4 ± 20.6	32 ± 16.8	0.20
IL-27	219 ± 74.4	95 ± 139	0.20
IP-10	978 ± 324	618 ± 113	0.23
MCP-3	2886 ± 1805	1248 ± 755	0.23
IL-18	7.9 ± 5.9	4.2 ± 3.1	0.24
MCP-1	13.3 ± 10.7	4.7 ± 2.1	0.27
IL-22	10.1 ± 5.6	7.2 ± 6.3	0.31
MIP-2	29.1 ± 8	36.8 ± 5.5	0.40
IL-4	8.3 ± 6.	5.3 ± 1.5	0.43
IL-5	11.3 ± 8.2	43.8 ± 41.6	0.50
IL-1 beta	1.8 ± 1.3	2.8 ± 2.1	0.59
MIP-1 beta	25 ± 8.2	29 ± 3.3	0.70
RANTES	285.2 ± 177	378 ± 55.8	0.70
IL-12p70	3.8 ± 1.5	3 ± 1.8	0.82
TNF-alpha	11.7 ± 7.5	8 ± 4.5	0.86
MIP-1 alpha	214.6 ± 58.3	226.8 ± 37.3	0.86

Figure 2. PUVA pretreatment influenced chemokine and Th1/Th2/Th17 cytokine profile expression. (**A**) Samples collected 24 hrs after the last IMQ treatment, (**B**) Effect on serum cytokines and chemokines due to PUVA pretreatment before IMQ exposure analyzed by 26-plex bead immunoassay. Data shown are from one representative experiment, with n=5 mice. Statistical analysis was performed using unpaired t-test or Mann–Whitney U test whichever appropriate

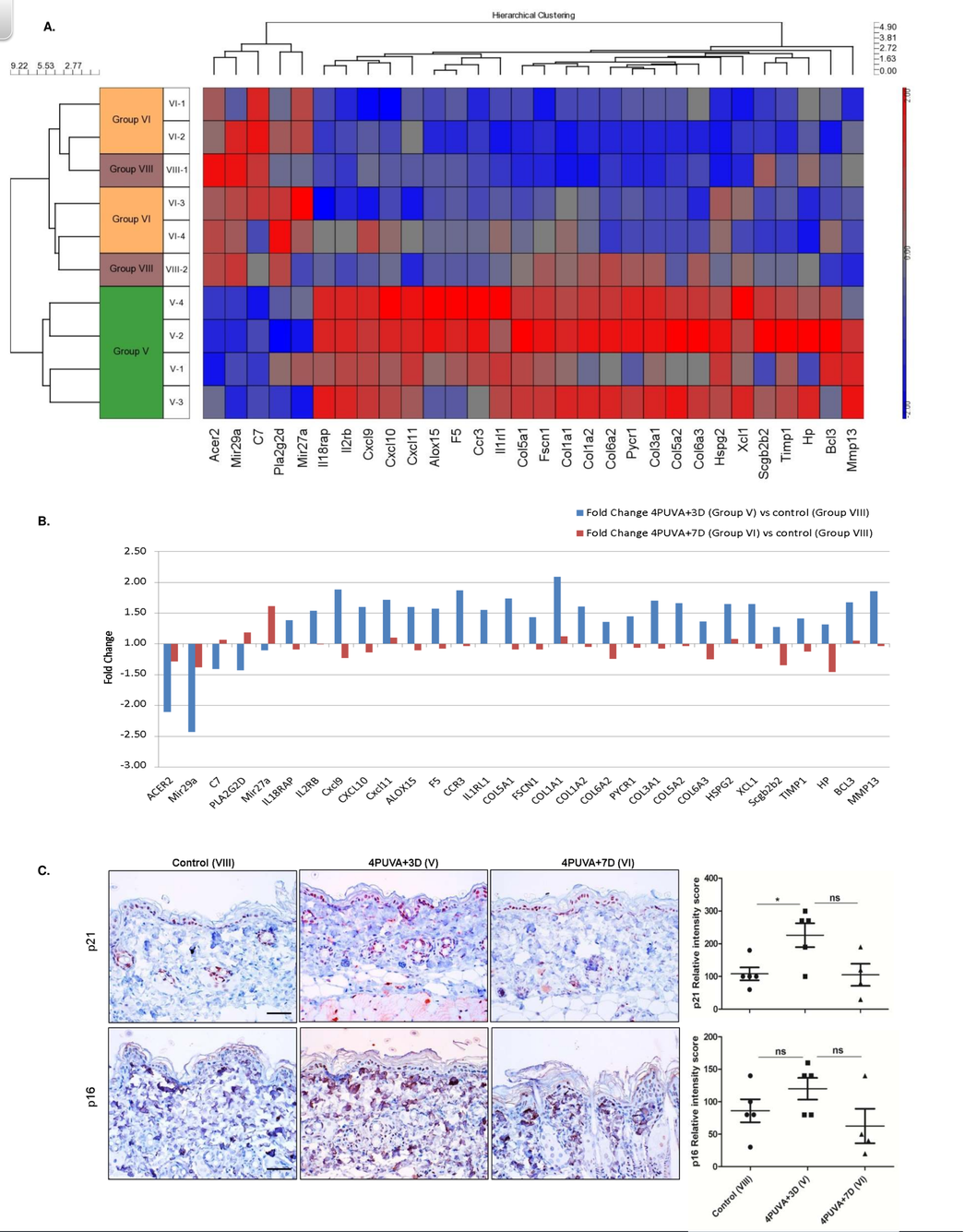
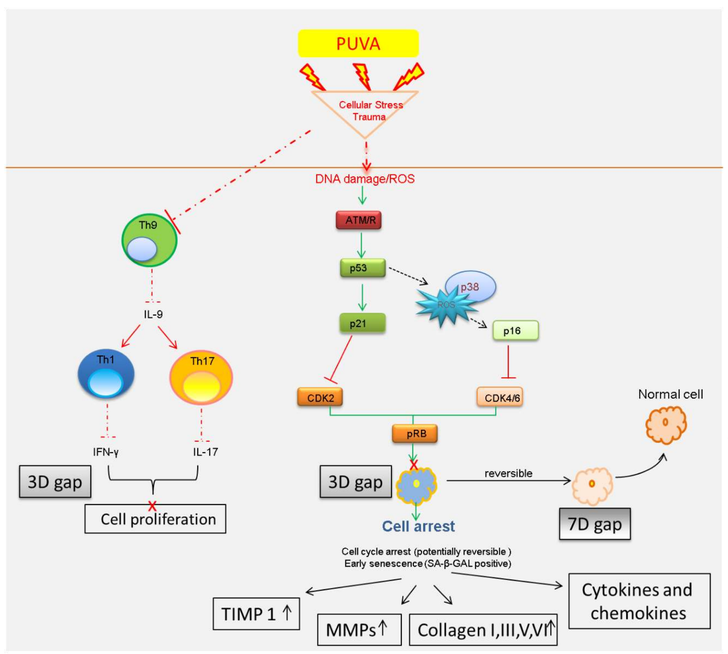


Figure 3. (**A**) Heat map of gene array expression with changes in molecular signature due to PUVA pretreatment. (A) Genes identified on the basis of the fold change of >1.5 and a p-value of <0.05 are plotted. Heat map of expression values for 30 genes is shown by two-way clustering of expression levels for Group V (4PUVA+3D) compared to Group VI (4PUVA+7D) where n=4 per group and Group VIII (Control) where n=2. Red denotes increased and blue denotes decreased expression levels. (**B**) Bar diagram indicates the fold changes in gene expression after PUVA pretreatment in comparison to controls (Group VIII). Statistical analysis for this data set was performed using the Partek Software v.6.6, as outlined in the M&M section.(**C**) Expression of p21/p16 in PUVA-pretreated skin with n=4-5 per group. Magnification 20X, scale bar 100 µm. Semi-quantitative scoring for p21 and p16 expression is expressed as relative intensity score, derived by multiplying the grade of nuclear intensity with the percentage of positive cells for p21 and p16 protein per sample and section. Statistical analysis was performed using unpaired t-test (*p < 0.05).

Summary



- ✓ PUVA to a greater degree than UVB was able to suppress the susceptibility of murine skin to mount a psoriatic inflammation
- ✓ This was paired with the enrichment of senescence pathway genes after PUVA pretreatment
- ✓ PUVA pretreatment did downregulate baseline levels (before IMQ treatment) of IFN-gamma, IL-17 and IL-9 cytokines that drive psoriatic inflammation

Figure 4. Schematic representation of the study hypothesis to understand the effects of phototherapy pretreatment leading to modulating the proinflammatory cytokines and extracellular matrix related genes expression, thereby priming the skin to be more tolerant to IMQ stimulation.

Conclusions

1. This findings indicate that PUVA primes the skin in such a manner as to shift the balance in favour of a reduced responsiveness to IMQ.
2. However this anti-psoriatic effect was lost when 7 days were elapsed between last PUVA exposure and start of IMQ, indicating that PUVA-induced senescence may be indeed crucial for its anti-psoriatic activity.

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