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Effect of cytokine and antigen stimulation on peripheral blood lymphocyte syndecan-1 expression

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Introduction: Cytokines are not only produced by activated lymphocytes but also interact with a number of cell-surface molecules on the same cells. Syndecan-1 is one such cell-surface molecule, which has the capacity to bind a variety of growth factors as well as cytokines. The aim of this study was to examine the effects of transforming growth factor β (TGF-β), interleukin-1 (IL-1), IL-2, IL-4, lipopolysaccharide (LPS) from *Porphyromonas gingivalis* and tetanus toxoid on syndecan-1 expression by B and T lymphocytes.
Methods: B and T lymphocytes were obtained from the peripheral blood of healthy donors. Following exposure to the above growth factors, cytokines and antigens, syndecan-1 expression was determined by flow cytometry.
Results: Subjects could be categorized as high or low expressors of syndecan-1. In the high-responder group TGF-β1 alone resulted in a significant increase in syndecan-1 expression by both B and T cells. None of the other cytokines and antigens produced a significant response. When analysed in combination, TGF-β1 in combination with IL-2, IL-4, *P. gingivalis* LPS and tetanus toxoid all produced significant increases in syndecan-1 expression by B cells. For T cells, combinations of TGF-β1 with IL-2 and tetanus toxoid resulted in increased syndecan-1 expression.
Conclusions: Both B and T lymphocytes synthesize the cell-surface proteoglycan syndecan-1 and its expression can be modulated by TGF-β1, either alone or in combination with IL-2, IL-4 and LPS from *P. gingivalis* and tetanus toxoid. While these may reflect general responses under inflammatory conditions their biological significance requires further investigation.

Key words: lymphocytes; periodontal disease; proteoglycans; syndecan-1

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It has been recognized for some time that biosynthesis of proteoglycans by T lymphocytes can be increased following mitogenic stimulation (3, 17, 29). In addition, more recent studies have suggested that cytokines secreted by T lymphocytes, either alone or as a result of direct contact between T and B lymphocytes, might regulate expression of the B-lymphocyte cell-surface proteoglycan syndecan-1 in periodontal inflammation (34).

Cytokines are produced by activated lymphocytes, monocytes and fibroblasts. They are released at sites of inflammation, act at short distances and can further induce inflammatory leukocyte infiltration (22, 23, 39, 44, 51). Many cytokines and growth factors possess proteoglycan-binding sites. Cytokines bound to proteoglycans on one cell can be presented to another cell in a paracrine fashion (19). Alternatively, cell-surface proteoglycans may capture cytokines and facilitate intracellular signalling, and modification of cell behaviour (21). In particular, syndecan-1 has been implicated as a regulator of cytokine function (14, 15).

Tetanus toxoid and lipopolysaccharide (LPS) extracted from *Porphyromonas*

gingivalis are well-recognized antigenic stimulants for T lymphocytes and B lymphocytes, respectively (31, 48). Both interleukin-2 (IL-2) and IL-4 are growth-mediating cytokines for T and B lymphocytes that signal through a receptor composed of specific α and β units and a common chain (5, 6, 13, 20, 27, 45, 50). IL-2 acts as a co-signalling factor, which supports initiation of cellular proliferation in non-activated lymphocytes, while in pre-activated T lymphocytes it is a potent survival factor, preventing apoptosis-facilitating action and sustained growth of T lymphocytes in an autocrine fashion (2, 33, 41). IL-4 is associated with T helper type 2 activation and with the growth and differentiation of activated B lymphocytes (35, 37, 42, 43, 47). Transforming growth factor-β1 (TGF-β1) is another important immunoregulator of cytokines in inflammation and tissue repair (36, 38). IL-1 is a pro-inflammatory cytokine produced at the site of inflammation (9, 10, 25, 30, 40).

In light of our previous observation that syndecan-1 expression is modulated under inflammatory conditions (34), the aim of the present study was to examine, in more detail, the effect of selected cytokines and antigens on syndecan-1 expression by peripheral blood lymphocytes *in vitro*.

Materials and methods

Subjects

Healthy volunteers aged 30 to 50 years (eight females and seven males) from the staff of the Dental School of the University of Queensland, were included in the study group. Ethics Committee approval and informed consent from all subjects participating in the study were obtained. A medical history and oral examination were conducted to rule out periodontal and systemic disease activity or medication use, which are known to influence the results of *in vitro* immunological examinations (11, 16).

Isolation of peripheral blood mononuclear cells

Under aseptic conditions, peripheral blood was taken by venepuncture from the median cephalic vein in the antecubital fossa. The blood was collected into 10-ml preservative-free Vacutainer tubes containing 143 USP units of lithium heparin (Becton Dickinson, North Ryde, Australia). All blood samples were collected as close as possible to 09.00 h to control for circadian variations in endogenous cortisol levels (16, 18). Within 30–60 min the samples were transported to the laboratory where all subsequent procedures were performed under aseptic conditions in a laminar flow bio-safety cabinet. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by density-gradient centrifugation (4). Finally, the PBMC were suspended in AIM-V serum-free medium (Gibco BRL, Life Technologies, Grand Island, NY).

PBMC stimulation

The PBMC were adjusted to 1×10^6 cells/ml and 2 ml aliquots were placed into six-well plates (Nunclon, Raskilde, Denmark). The PBMC were unstimulated or were stimulated with IL-2 (50 or 500 U/ml); IL-4 (5 or 50 ng/ml); *P. gingivalis* LPS (5 or 10 μg/ml); recombinant human TGF-β1 (1, 2 or 4 ng/ml); thymol-free tetanus toxoid (TT) (10 or 20 μg/ml); IL-1α (15 or 30 pg/ml); IL-1β (1 ng/ml); TGF-β1 (4 ng/ml) plus IL-2 (50 U/ml); TGF-β1 (4 ng/ml) plus IL-4 (50 ng/ml); TGF-β1 (4 ng/ml) plus *P. gingivalis* LPS (5 μg/ml); or TGF-β1 (4 ng/ml) plus TT (20 μg/ml). The PBMC were cultured for 7 days at 37°C in a humidified atmosphere of 5% carbon dioxide. In parallel, PBMC were cultured for 4 days in the presence of TGF-β1 (4 ng/ml), TGF-β1 (2 ng/ml), or IL-1β (30 pg/ml) or for 24 h in the presence of TGF-β1 (4 ng/ml). The time and concentration were optimized based on the study (data not shown) conducted on peripheral blood lymphocyte samples taken from healthy individuals on day zero, unstimulated (control) and stimulated on days 3, 5 and 7.

Flow cytometry

At the end of the culture period, the cells were washed twice with cold phosphate-buffered saline containing 0.1% sodium azide. Non-specific antibody binding was blocked with 10% mouse serum for 30 min. Cells were incubated for 30 min at 40°C in the dark with a 1 : 50 dilution of monoclonal anti-CD3 conjugated with peridinin–chlorophyll protein complex (PerCP) (Pharmingen, San Diego, CA) specific for T lymphocytes, fluorescein isothiocyanate-conjugated anti-CD19 (Dako, Botany, Australia) specific for B lymphocytes and anti-CD138-R-Phycoerythrin (RPE) or phycoerythrin-conjugated MCA-681 (Serotech Inc., Oxford, UK) specific for syndecan-1. For the negative controls, equal numbers of cells were incubated with PerCP, fluorescein isothiocyanate and RPE mouse immunoglobulin G1 (Pharmingen). Cells were washed twice with cold phosphate-buffered saline containing 0.1% sodium azide and suspended in 100 μl fixative containing 1% sodium azide and 10% formaldehyde (Becton Dickinson, North Ryde, Australia). Flow cytometric analysis was undertaken on the same day (FACScaliber, Becton Dickinson, San Jose, CA). Data were collected for a minimum of 10,000 cells gated on forward and side scatters and analyzed using CellQuest software (Becton Dickinson). Markers were positioned to include 95% of the isotype control cells in the negative control quadrant.

Statistical analyses

Analysis of variance and multiple regression analysis were undertaken using Graph Pad Instat and Microsoft Excel. Results were considered significant at $P < 0.05$.

Results

TGF-β1 stimulation

Subjects were divided arbitrarily into those showing relatively high syndecan-1 expression on stimulation and those showing relatively low syndecan-1 expression with TGF-β1 at different concentrations for 7 days (Table 1). The relative increase or decrease in syndecan-1 expression following stimulation was calculated based on the difference from the respective unstimulated (control) percentage of peripheral blood lymphocyte syndecan-1 expression. The subjects were grouped, based on the results, as high responders or low responders. Following stimulation with TGF-β1 at a concentration of 4 ng/ml, 78.9% were high responders while 21.1% were low responders. There was a highly significant increase in syndecan-1 expression in positive responders following stimulation with TGF-β1 (4 ng/ml) for both the peripheral blood T and B lymphocytes ($P = 0.009$ and $P = 0.0005$, respectively). In relation to stimulation with TGF-β1 at a concentration of 2 ng/ml, 62.5% of the subjects were high responders and 37.5% were low responders. Unlike the higher concentrations, following stimulation with TGF-β1 at a concentration of 1 ng/ml, 54% of subjects showed decreased syndecan-1 expression in the peripheral blood T and B lymphocytes in relation to the controls ($P = 0.07$ and $P = 0.04$, respectively).

Following stimulation with TGF-β1 (4 and 2 ng/ml) for 4 days, PBMC showed a marginal, but insignificant, increase in syndecan-1 expression (Fig. 1). Even when cultured for shorter time periods

Table 1. The percentage of T and B lymphocytes expressing syndecan-1 at various concentrations of transforming growth factor-β1 (TGF-β1)

	High responders		Low responders	
	T cells	B cells	T cells	B cells
Unstimulated	8.4 ± 4.8	32.8 ± 4.6	10.41 ± 3.3	30 ± 3.8
TGF-β1 (4 ng/ml)	14.38 ± 5.4	61.43 ± 3.6	7.5 ± 0.5	24 ± 5.1
Unstimulated	9.31 ± 3.12	34.69 ± 7.3	5.9 ± 3.9	24.8 ± 7.6
TGF-β1 (2 ng/ml)	18.99 ± 3.9	53.21 ± 6.7	7.9 ± 4.01	21.8 ± 4.7
Unstimulated	8.35 ± 4.9	43.69 ± 5.6	10.9 ± 6.06	50.36 ± 4.9
TGF-β1 (1 ng/ml)	10.61 ± 3.8	56.7 ± 13.5	6.1 ± 4.3	33 ± 5.8

Data are presented as the means and standard deviations of the mean for triplicate samples. The TGF-β1 concentrations (4, 2 and 1 ng/ml) of 7-day duration, based on the increase or decrease in the percentage lymphocyte syndecan-1 expression from the baseline (unstimulated) have been grouped as high responders and low responders.

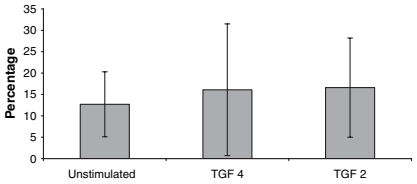

Fig. 1. Percentage of peripheral blood T and B lymphocytes expressing syndecan-1, following stimulation of peripheral blood mononuclear cells with transforming growth factor-β1 (TGF-β1; 4 and 2 ng/ml) for 4 days. Data are presented as the means and standard deviations.

such as 24 h in the presence of TGF-β1 (4 ng/ml), PBMC showed no increase in syndecan-1 expression in relation to the control cultures (Fig. 2).

Other cytokine stimulation

Stimulation with the individual cytokines IL-2, IL-4, IL-1α, and IL-1β resulted in a trend towards decreased syndecan-1 expression by peripheral blood T and B lymphocytes. However, the values failed to reach statistical significance compared to the controls because of wide individual variations (Table 2).

Antigen stimulation

Following stimulation with tetanus toxoid (TT) and *P. gingivalis* LPS, 26.6% and 35% of the subjects, respectively, were identified as positive responders. The expression of syndecan-1 by B and T lymphocytes exposed to the antigens for 7 days is presented in Table 3. Stimulation with TT and LPS resulted in a general increase in syndecan-1 expression by both subsets of lymphocytes. However, this was significant only for the high responder B cells.

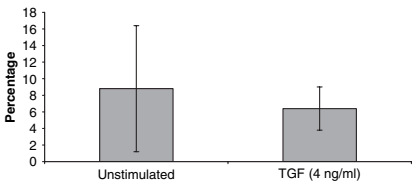

Fig. 2. Percentage of peripheral blood T and B lymphocytes expressing syndecan-1, following stimulation of peripheral blood mononuclear cells with transforming growth factor-β1 (TGF-β1; 4 ng/ml) for 24 h. Data are presented as means and standard deviations.

TGF-β1 combined, cytokine/antigen stimulation

An investigation using combinations of cytokines and antigens on the percentage of peripheral blood B lymphocytes expressing syndecan-1 indicated an increase in syndecan-1 expression following stimulation with various combinations of growth factor and cytokines/antigens. Compared to the unstimulated controls, TGF-β1 in combination with IL-2, IL-4, *P. gingivalis* LPS and TT all showed significant increases in syndecan-1 synthesis ($P < 0.05$) (Fig. 3). However, for the peripheral blood T lymphocytes expressing syndecan-1, statistical significance was observed only for combinations of TGF-β1 with IL-2 and TT compared with control (unstimulated) (Fig. 4). For both T and B cells the addition of cytokines to TGF-β1 appeared to have an additive effect.

Discussion

From this study we have determined that both B and T lymphocytes synthesize the cell surface proteoglycan syndecan-1 *in vitro* and that its expression can be modulated by TGF-β1, either alone or in combination with IL-2, IL-4 and specific antigens such as LPS from *P. gingivalis* and TT. Stimulation of syndecan-1 expression by B and T lymphocytes may have some beneficial effects in immune regulation through the binding of cytokines and growth factors and in the control of lymphocyte migration. One cytokine that significantly affects lymphocyte function is TGF-β1 (24, 36).

With regards to B lymphocytes, TGF-β1 is an important autocrine immunoregulatory molecule limiting clonal expansion and differentiation of normal B lymphocytes, regulating the development from the pre-B-cell stage through to immunoglobulin-

Table 2. The percentage of T and B lymphocytes expressing syndecan-1 following cytokine stimulation of peripheral blood mononuclear cells

	T lymphocytes	B lymphocytes
7 days unstimulated	8.391 ± 3.71	33.24 ± 11.65
IL-2 (50 U/ml)	7.81 ± 2.81	23.04 ± 7.17
IL-2 (500 U/ml)	8.54 ± 3.15	23.51 ± 1.29
IL-4 (5 ng/ml)	9.03 ± 3.94	27.81 ± 10.87
IL-4 (50 ng/ml)	7.75 ± 4.21	25.85 ± 4.31
IL-1α (1 ng/ml)	5.67 ± 2.46	27.81 ± 18.64
IL-1β (30 pg/ml)	5.48 ± 1.64	21.52 ± 1.41
IL-1β (15 pg/ml)	5.81 ± 3.13	31.88 ± 10.05
4 days unstimulated	15.02 ± 4.886	34.02 ± 1.91
IL-1β (30 pg/ml)	13.39 ± 2.19	35.19 ± 12.32

Data are presented as the means and standard deviations of the mean for triplicate samples.

Table 3. The percentage of T and B lymphocytes expressing syndecan-1, following tetanus toxoid (TT) and *Porphyromonas gingivalis* lipopolysaccharide (LPS) stimulation of peripheral blood mononuclear cells in positive and negative responders

	Positive responders		Negative responders	
	T lymphocytes	B lymphocytes	T lymphocytes	B lymphocytes
Unstimulated	4.583 ± 2.42	36.38 ± 14.82	10.12 ± 3.81	52.19 ± 5.38
TT (10 μg/ml)	7.53 ± 5.1	45.29 ± 16.2	6.36 ± 2.12	34.01 ± 14.01
TT (20 μg/ml)	10.34 ± 7.03	37.38 ± 3.98	n.d.	n.d.
Unstimulated	7.13 ± 3.7	56.73 ± 12.82	10.96 ± 4.3	47.61 ± 13.72
LPS (5 μg/ml)	12.48 ± 5.6	73.64 ± 13.63	8.87 ± 5.98	38.39 ± 15.38
LPS (10 μg/ml)	n.d.	n.d.	11.8 ± 3.48	49.26 ± 20.68

Data are presented as the means and standard deviations of the mean for triplicate samples.
n.d., not determined.

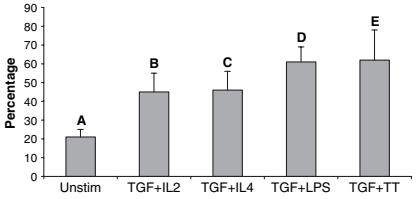

Fig. 3. Percentage of peripheral blood B-lymphocytes expressing syndecan-1, following stimulation of peripheral blood mononuclear cells for 7 days (A) unstimulated or control; (B) TGF-β1 (4 ng/ml) + IL-2 (50 U); (C) TGF-β1 (4 ng/ml) + IL-4 (5 ng/ml), $*P = 0.03$; (D) TGF-β1 (4 ng/ml) + *Porphyromonas gingivalis* LPS (5 μg/ml), $***P = 0.0008$; (E) TGF-β1 (4 ng/ml) + TT (10 μg/ml), $*P = 0.03$. Data are presented as the means and standard deviations. Abbreviations: interleukin-2 (IL-2), lipopolysaccharide (LPS), tetanus toxoid (TT), transforming growth factor-β1 (TGF-β1).

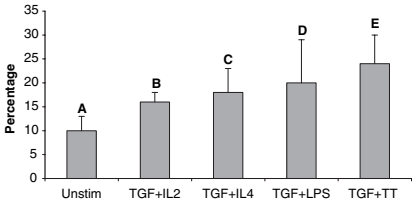

Fig. 4. Percentage of peripheral blood T lymphocytes expressing syndecan-1, following stimulation of peripheral blood mononuclear cells for 7 days (A) unstimulated or control; (B) TGF-β1 (4 ng/ml) + IL-2 (50 U); (C) TGF-β1 (4 ng/ml) + IL-4 (5 ng/ml), $*P = 0.05$; (D) TGF-β1 (4 ng/ml) + *Porphyromonas gingivalis* LPS (5 μg/ml); (E) TGF-β1 (4 ng/ml) + TT (10 μg/ml). Data are presented as the means and standard deviations. Abbreviations: interleukin-2 (IL-2), lipopolysaccharide (LPS), tetanus toxoid (TT), transforming growth factor-β1 (TGF-β1).

secreting plasma cells and apoptosis (8, 22, 28, 32, 49). Since TGF-β1 affects syndecan-1 synthesis, this may imply that syndecan-1 is associated with B-lymphocyte differentiation and maturation (46).

In general, the effects of TGF-β1 on T lymphocyte populations are indirect, rather than direct, via altered expression of cell surface receptors or secretion of various cytokines (7). Thus, cell differentiation and isotype switching of activated cells may be associated with variable expression of cell surface molecules such as syndecan-1 (1, 46, 52, 53). However, in the present study, IL-2 and IL-4, (known lymphocyte growth factor cytokines) failed to cause any significant increase in syndecan-1 expression. While this may have been the result of individual variations, IL-2 and IL-4 are recognized to result in both synergy and antagonism of cellular signalling processes in both T and B lymphocytes (5). As for IL-2 and IL-4, short-term exposure of peripheral blood B and T lymphocytes to IL-1β also failed to affect syndecan-1 expression. However, following 7 days of stimulation, IL-1β tended to cause a decrease in syndecan-1 expression in both the T-lymphocyte and B-lymphocyte populations, although this did not reach significance compared to the control.

Stimulation with LPS from *P. gingivalis* and TT resulted in peripheral blood B and T lymphocytes showing a variable increase in syndecan-1 expression, compared to the controls. The variation in syndecan-1 expression was more prominent for B lymphocytes following antigen stimulation than for T lymphocytes. This may reflect differences in antigen presentation to B and T lymphocytes, respectively. The increase in syndecan-1 expression following exposure to LPS in B lymphocytes may correspond with the observations of syndecan-1 expression in advanced periodontitis (34).

When cytokines and antigens were combined with TGF-β, the percentage of peripheral blood B lymphocytes expressing syndecan-1 increased significantly. Although there was an increased syndecan-1 expression by peripheral blood T lymphocytes following stimulation with TGF-β1 and IL-4, but not LPS, the B lymphocytes showed an increase in syndecan-1 for TGF-β1 with both LPS and IL-4. TGF-β1 may act as a synergistic factor in combination with IL-4, and may result in IL-4-triggered antigen activation, up-regulated syndecan-1 expression and B-lymphocyte activation. In the case of B lymphocytes, TGF-β1 and IL-4 have been shown to have a synergistic effect, as they may be acting through selectively inhibiting the growth of immunoglobulin M-positive B lymphocytes, which allows immunoglobulin A-positive cells to outgrow (26). This leads to the question of whether syndecan-1 expression is related to subset variations and/or differentiation of lymphocytes independent of cell proliferation, as alluded to in some studies (3).

Increased syndecan-1 expression by peripheral blood B lymphocytes following stimulation with combinations of TGF-β1, IL-4 and *P. gingivalis* LPS may mimic, in part, the inflammatory reactions seen in periodontitis. The combination of TGF-β1 and *P. gingivalis* LPS led to an increase in syndecan-1 expression by peripheral blood B lymphocytes but no significant variation in syndecan-1 expression by peripheral blood T lymphocytes. This may be associated with changes in T lymphocytes to a T helper type 2 subtype, which in turn may lead to B-lymphocyte activation as seen in chronic periodontitis. TGF-β1 appears to enhance LPS-stimulated isotype switching in B lymphocytes and results in a significant increase in immunoglobulin A secretion (26). TGF-β1 and TT also showed an increased percentage of peripheral blood B lymphocytes positive for syndecan-1 expression. This suggests that specific T-lymphocyte subsets may influence activation of B lymphocytes because the TT is a T-lymphocyte-specific antigen. Alternatively, TT may have enhanced a suppressive function of TGF-β1 on specific T-lymphocyte subsets, favouring B-lymphocyte activation (12). The significance of subset variation in relation to the cytokine-influenced syndecan-1 up-regulation should be explored to investigate its relationship to pathogenic profiles as has been suggested by other reports concerning the role of syndecan-1 in inflammatory conditions (14, 15).

In conclusion, this study has shown that TGF-β alone and/or in combination with other cytokines and antigens enhances syndecan expression. This is a necessary starting point; further studies are now needed to determine the mechanism of this enhancement. In addition, the biological significance of this remains speculative and requires further investigation.

References

1. Angelin-Duclos C, Cattoretti G, Lin KI, Calame K. Commitment of B-lymphocytes to a plasma cell fate is associated with Blimp-1 expression *in vivo*. J Immunol 1984: **165**: 5462–5471.
2. Armant M, Delespesse G, Sarfati M. IL-2 and IL-7 but not IL-12 protect natural killer cells from death by apoptosis and up-regulate bcl-2 expression. Immunology 1995: **85**: 331–337.
3. Bartold PM, Haynes DR, Vernon-Roberts B. Effect of mitogen and lymphokine stimulation on proteoglycan synthesis by lymphocytes. J Cell Physiol 1989: **140**: 82–90.
4. Böyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968: **21** (suppl 97): 77–89.
5. Burke MA, Morel BF, Oriss TB, Bray J, McCarthy SA, Morel PA. Modeling the proliferative response of T-cells to IL-2 and IL-4. Cell Immunol 1997: **178**: 42–52.
6. Cantrell DA, Smith KA. The interleukin-2 T-lymphocyte system: a new cell growth model. Science 1984: **224**: 1312–1316.
7. Cerwenka A, Swain SL. TGF-beta1: immunosuppressant and viability factor for T-lymphocytes. Microbes Infect 1999: **1**: 1291–1296.
8. Chaouchi N, Arvanitakis L, Auffredou MT, Blanchard DA, Vazquez A, Sharma S. Characterization of transforming growth

factor-beta 1 induced apoptosis in normal human B-cells and lymphoma B cell lines. Oncogene 1995: **11**: 1615–1622.
9. Durum SK, Schmidt JA, Oppenheim JJ. Interleukin 1: an immunological perspective. Annu Rev Immunol 1985: **3**: 263–287.
10. Engebretson SP, Lamster IB, Herrera-Abreu M et al. The influence of interleukin gene polymorphism on expression of interleukin-1beta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. J Periodontol 1999: **70**: 567–573.
11. Evans RI, Mikulecky M, Seymour GJ. Effect of initial treatment of chronic inflammatory periodontal disease in adults on spontaneous peripheral blood lymphocyte proliferation. J Clin Periodontol 1989: **16**: 271–277.
12. Fox FE, Ford HC, Douglas R, Cherian S, Nowell PC. Evidence that TGF-beta can inhibit human T-lymphocyte proliferation through paracrine and autocrine mechanisms. Cell Immunol 1993: **150**: 45–58.
13. Gillis S, Watson J. Biochemical and biological characterization of lymphocyte regulatory molecules. V. Identification of an interleukin 2-producing human leukemia T cell line. J Exp Med 1980: **152**: 1709–1719.
14. Götte M. Syndecans in inflammation. FASEB J 2003: **17**: 575–591.
15. Götte M, Echtermeyer F. Syndecan-1 as a regulator of chemokine function. Sci World J 2003: **3**: 1327–1331.
16. Hahn BH, Macdermott RP, Jacobs SB, Pletscher LS, Beale MG. Immunosuppresive effects of low doses of glucocorticoids: effects on autologous and allogenic mixed leukocyte reactions. J Immunol 1980: **124**: 2812–2817.
17. Hart GW. Biosynthesis of glycosaminoglycans by thymic lymphocytes. Effects of mitogenic activation. Biochemistry 1982: **21**: 6088–6096.
18. Indiveri F, Pierri I, Rogna S et al. Circadian variations of autologus mixed lymphocyte reactions and endogenous cortisol. J Immunol Methods 1985: **82**: 17–24.
19. Jalkanen ST, Bargatze RF, Herron LR, Butcher EC. A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. Eur J Immunol 1986: **16**: 1195–1202.
20. Johnston JA, Wang LM, Hanson EP et al. Interleukins 2, 4, 7, and 15 stimulate tyrosine phosphorylation of insulin receptor substrates 1 and 2 in T-cells. Potential role of JAK kinases. J Biol Chem 1995: **270**: 28527–28530.
21. Kainulainen V, Nelimarkka L, Jarvelainen H, Laato M, Jalkanen M, Elenius K. Suppression of syndecan-1 expression in endothelial cells by tumor necrosis factor-alpha. J Biol Chem 1996: **271**: 18759–18766.
22. Kehrl JH, Roberts AB, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS. Transforming growth factor beta is an important immunomodulatory protein for human B-lymphocytes. J Immunol 1986: **137**: 3855–3860.
23. Kishimoto TK, Springer TA. Human leukocyte adhesion deficiency: molecular basis for a defective immune response to infections of the skin. Curr Probl Dermatol 1989: **18**: 106–115.
24. Kondo S, Isobe K, Ishiguro N, Nakashima I, Miura T. Transforming growth factor-beta 1 enhances the generation of allospecific cytotoxic T-lymphocytes. Immunology 1993: **79**: 459–464.
25. Kornman KS, Crane A, Wang HY et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997: **24**: 72–77.
26. Kunimoto DY, Ritzel M, Tsang M. The roles of IL-4, TGF-beta and LPS in IgA switching. Eur Cytokine Netw 1992: **3**: 407–415.
27. Lai SY, Molden J, Liu KD, Puck JM, White MD, Goldsmith MA. Interleukin-4-specific signal transduction events are driven by homotypic interactions of the interleukin-4 receptor alpha subunit. EMBO J 1996: **15**: 4506–4514.
28. Lebman DA, Edmiston JS. The role of TGF-beta in growth, differentiation, and maturation of B-lymphocytes. Microbes Infect 1999: **1**: 1297–1304.
29. Levitt D, Ho PL. Induction of chondroitin sulfate proteoglycan synthesis and secretion in lymphocytes and monocytes. J Cell Biol 1983: **97**: 351–358.
30. Lloyd AR, Oppenheim JJ. Poly's lament: the neglected role of the polymorphonuclear neutrophil in the afferent limb of the immune response. Immunol Today 1992: **13**: 169–172.
31. Loesche WJ, Syed SA, Schmidt E, Morrison EC. Bacterial profiles of subgingival plaques in periodontitis. J Periodontol 1985: **56**: 447–456.
32. Lomo J, Blomhoff HK, Beiske K, Stokke T, Smeland EB. TGF-beta 1 and cyclic AMP promote apoptosis in resting human B-lymphocytes. J Immunol 1995: **154**: 1634–1643.
33. Lorenz HM, Hieronymus T, Grunke M, Manger B, Kalden JR. Differential role for IL-2 and IL-15 in the inhibition of apoptosis in short-term activated human lymphocytes. Scand J Immunol 1997: **45**: 660–669.
34. Manakil JF, Li H, Sugerman PB, Seymour GJ, Bartold PM. The expression of cell surface proteoglycans by lymphocytes from peripheral blood and gingiva in health and periodontal disease. J Dent Res 2001: **80**: 1704–1710.
35. Miller CL, Hooton JW, Gillis S, Paetkau V. IL-4 potentiates the IL-2-dependent proliferation of mouse cytotoxic T-cells. J Immunol 1990: **144**: 1331–1337.
36. Miyazono K. TGF-beta receptors and signal transduction. Int J Hematol 1997: **65**: 97–104.
37. Moreau JL, Chastagner P, Tanaka T et al. Control of the IL-2 responsiveness of B-lymphocytes by IL-2 and IL-4. J Immunol 1995: **155**: 3401–3408.
38. Ohtsuka K, Gray JD, Stimmler MM, Toro B, Horwitz DA. Decreased production of TGF-beta by lymphocytes from patients with systemic lupus erythematosus. J Immunol 1998: **160**: 2539–2545.
39. Oppenheimer-Marks N, Davis LS, Lipsky PE. Human T-lymphocyte adhesion to endothelial cells and transendothelial migration. Alteration of receptor use relates to the activation status of both the T cell and the endothelial cell. J Immunol 1990: **145**: 140–148.
40. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000 2000: **14**: 216–248.
41. Pericle F, Giovarelli M, Colombo MP et al. An efficient Th2-type memory follows CD8+ lymphocyte-driven and eosinophil-mediated rejection of a spontaneous mouse mammary adenocarcinoma engineered to release IL-4. J Immunol 1994: **153**: 5659–5673.
42. Powrie F, Coffman RL, Correa-Oliveira R. Transfer of CD4+ T-cells to C.B-17 SCID mice: a model to study Th1 and Th2 cell differentiation and regulation *in vivo*. Res Immunol 1994: **145**: 347–353.
43. Rudolphi A, Enssle KH, Claesson MH, Reimann J. Adoptive transfer of low numbers of CD4+ T-cells into SCID mice chronically treated with soluble IL-4 receptor does not prevent engraftment of IL-4-producing T-cells. Scand J Immunol 1993: **38**: 57–64.
44. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. Science 1987: **238**: 491–497.
45. Ruscetti FW, Gallo RC. Human T-lymphocyte growth factor: regulation of growth and function of T-lymphocytes. Blood 1981: **57**: 379–394.
46. Sanderson RD, Lalor P, Bernfield M. B-lymphocytes express and lose syndecan at specific stages of differentiation. Cell Regul 1989: **1**: 27–35.
47. Scott P. Selective differentiation of CD4+ T helper cell subsets. Curr Opin Immunol 1993: **5**: 391–397.
48. Slots J. *Actinobacillus actinomycetemcomitans* and *Bacteroides gingivalis* in advanced periodontitis in man. Dtsch Zahnarztl Z 1984: **39**: 615–622.
49. Smeland EB, Blomhoff HK, Holte H et al. Transforming growth factor type beta (TGF beta) inhibits G1 to S transition, but not activation of human B-lymphocytes. Exp Cell Res 1987: **171**: 213–222.
50. Smith KA, Baker PE, Gillis S, Ruscetti FW. Functional and molecular characteristics of T-lymphocyte growth factor. Mol Immunol 1980: **17**: 579–589.
51. Springer TA. The sensation and regulation of interactions with the extracellular environment: the cell biology of lymphocyte adhesion receptors. Annu Rev Cell Biol 1990: **6**: 359–402.
52. Turner CA, Jr, Mack DH, Davis MM. Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B-lymphocytes into immunoglobulin-secreting cells. Cell 1994: **77**: 297–306.
53. Yamashita Y, Oritani K, Miyoshi EK, Wall R, Bernfield M, Kincade PW. Syndecan-4 is expressed by B lineage lymphocytes and can transmit a signal for formation of dendritic processes. J Immunol 1999: **162**: 5940–5948.