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CYCLOSPORINE ENHANCES LIVER REGENERATION: THE ROLE OF HEPATOCYTE MHC EXPRESSION AND PGE_2 - A STUDY RELEVANT TO GRAFT IMMUNOGENICITY

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Abstract

Aim: We have investigated CsA induced liver hyperplasia to explore the potential effects on the immunogenicity of the regenerating liver within the clinical context of rejection after transplantation.

Materials and Methods: Flow cytometry analysis of hepatocytes, isolated 48 hours after 2/3 partial hepatectomy (PH2/3) or sham operation in rats, was performed to determine the effect of CsA on DNA synthesis and MHC molecule expression. The possible role of PGE_2 was evaluated by the administration of SC-19220, an EP_1PGE_2 receptor antagonist.

Results: CsA augmented liver regeneration and this was partially attenuated by SC-19220. The moderate expression of class I MHC expression, as well as the very low class II MHC expression detected in normal hepatocytes by flow cytometry was augmented after PH2/3 and reduced by CsA. The CsA-mediated decrease of hepatocyte immunogenicity was not SC-19220 dependent.

Conclusions: It is proposed that the enhancing effect of CsA on hepatocyte proliferation is by means of an indirect mechanism that can be attributed to a) reduced immunogenicity of the regenerating liver as a result of inhibition of class I and II MHC hepatocyte expression and b) increased PGE_2 synthesis in the liver mediated by its action on EP_1 receptor.

Key words: Liver regeneration; cyclosporine; immunogenicity: major histocompatibility complex; hepatocyte, hepatectomy; rats; prostaglandin E_2; SC-19220; flow cytometry.

Abbreviations: APC = antigen presenting cell, CsA = cyclosporine, MHC = major histocompatibility complex, PGE_2 = prostaglandin E_2, PH 2 / 3 = 2/3 partial hepatectomy, SC-19220 = l-acetyl-2-8-dihydrodibenz [b,f][l,4] oxazepine-10-carbonyl hydrazine

1. INTRODUCTION

The capacity of the liver to regenerate is considered to be vital in liver transplantation, particularly in split and living related segmental grafts. This regenerative response is influenced, at least in part, by immunosuppressive drugs [1]. The specific effect of CsA on liver regeneration has been studied in the experimental model of PH 2 / 3 in rats [2], a model believed to be comparable to the regenerative response after transplantation [3, 4]. However, various studies have reported conflicting results. Some have observed an enhancing effect [5-9], others an inhibitory effect [10], while still others described no effect at all [11]. It has been proposed that CsA augments liver regeneration by inhibiting the expression of the IL-2 gene in $CD4^+$ helper T lymphocytes, and thus preventing the T cell mediated immune reaction to the regenerating liver [5, 6,11, 12, 13]. This view is supported by previous findings in the experimental model of PH 2 / 3 in the rat, according to which liver regeneration is controlled by immume mechanisms [14, 15]. On the other hand, it was also found that the immunophilins (CsA and FK506) augment liver regeneration in the athymic NIH - RNU rats with congenital absence of T lymphocytes [16]. Moreover, the augmenting effect of CsA on liver regeneration can not be attributed to its other important mechanism of action, the enhancing of the expression of the TGF-β gene [17], known to be the main inhibitory factor of liver regeneration in vivo and of hepatocyte proliferation in primary culture [18, 19].

The experimental findings described so far lead to a dead end in the correlation between CsA and liver regeneration. A recent theory however, proposed that CsA affects antigen presenting cells (APCs) by reducing their ability to present antigens to T cells. This effect has been attributed to two possible mechanisms of action of CsA [20]: a) the direct inhibition of the expression of class I and II MHC molecules on APCs [21] and b) its augmenting action on PGE_2 production [22]. PGE_2 inhibits the expression of class I and II MHC molecules on APCs [23], which in turn reduces their ability to present antigens to T cells [24]. The possibility that CsA intervenes in liver regeneration through its effects on APCs is interesting because hepatocytes have the capacity to function as APCs [25, 26]. Proliferating hepatocytes stimulate the immune response during liver regeneration, and become themselves targets of cytotoxic immune pathways [27, 28].

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The augmenting effect of CsA on PGE_2 production by hepatocytes or other liver APCs could directly increase liver regeneration, in addition to its immunosuppressive action. More specifically, the augmenting role of PGE_2 on liver regeneration is supported by previous studies that show a rise of PGE_2 in regenerating liver and plasma [29], a reduction of liver regeneration after treatment with inhibitors of prostaglandin synthesis [30, 31], an enhancing effect of PGE_2 on DNA synthesis by cultured hepatocytes [29, 32-35], PGE_2 as an inducer of HGF gene expression [36], and PGE_2 as a participant in the mechanism of action of mitogens of liver regeneration such as HGF [32], EGF and TGFα [35, 37]. The aforementioned actions are attributed to the direct action on EP_1, EP_2 and EP_3 receptors of PGE_2 [38], which are expressed by hepatocytes [39].

We attempted to elucidate the mechanism of action of CsA on liver regeneration after 2/3 partial hepatectomy in wistar rats by studying : a) the effect of CsA on liver regeneration, b) the effect of CsA on the expression of class I and II MHC molecules by regenerating hepatocytes and c) the effect of SC-19220, a PGE_2 receptor antagonist of the EP_1 subtype [35, 40] on liver regeneration in the presence of CsA.

2. Materials and Methods

2.1 Materials

5'-bromo-2'-deoxyuridine (BrdUrd) was supplied by Boehringer Mannheim (Mannheim, Germany). CsA was supplied by Novartis (Basel, Switzerland). Olive oil was obtained from Elais (Athens, Greece). Cefuroxime was supplied by Glaxo (Bristol, UK). SC-19220 (l-acetyl-2-8-dihydrodibenz [b,f][l,4] oxazepine-10-carbonyl hydrazine) was purchased from Searle (Skokie, IL, USA). Osmotic minipumps, Alzet model 2ML1 were purchased from Alza (Palo Alto, CA. USA). Ether was obtained from Riedel-de Haen (Frankfurt, Germany). Heparin was supplied by Pharmacia (Uppsala, Sweden). Absolute alcohol, methanol, acetone, formaldehyde (4%), Leibowitz, hematoxylin, eosin, trypan blue and paraffin were all purchased from Gibco (Grand Island, NY, USA). DMSO, NaCl, KC1, $CaCl_2$, KH_2P0_4, $MgS0_4.7H_20$, $NaHCO_3$, HCl, EDTA, HBSS, type IV collagenase. PBS, pepsin and Tween 20 were all obtained from SIGMA (St Louis, MO, USA). Fluorescein isothiocyanate (FITC) - conjugated mouse anti-rat monoclonal antibodies OX-18 and OX-6 were supplied by Serotec (Bicester, Oxfordshire, UK). FITC-conjugated anti-rat monoclonal antibody against BrdUrd was purchased from Becton-Dickinson (Mountain View, CA, USA).

Vehicle A, for the intraperitoneal injection of SC-19220, was comprised of 0.2 ml DMSO, 15 mg cefuroxime, and 2 ml 0.9% NaCl. Vehicle B, for the dilution of SC-19220 in the osmotic minipumps, was comprised of 0.6 ml DMSO, 0.2 ml absolute alcohol, 7.5 mg of cefuroxime and 1.2 ml of 0.9% NaCl. Modified Krebs-Henseleit bicarbonate buffer solution was comprised of 118.41 mM NaCl, 4.75 mM KC1, 2.57 mM $CaCl_2$, 1.19 mM KH_2P0_4, 1.185 mM $MgS0_4.7H_20$ and 25 mM $NaHC0_3$ in sterile H_20. Modified HBSS solution was comprised of 50 mM EDTA in HBSS. Alcoholic formalin solution was comprised of 50% absolute alcohol and 50% formaldehyde solution.

2.2 Animals

Specified pathogen free, adult, male Wistar rats, weighing 200 - 250 gm (Department of Biology, Aristotle University, Thessaloniki, Greece) were housed under standard laboratory conditions with free access to food (rat pellet chow 510, ELVIZ, Thessaloniki, Greece) and water. All animals were acclimated for 2 weeks prior to experimentation. This study was approved by the local committee for care and use of laboratory animals.

2.3 Group Assignment

All animals received 100 mg BrdUrd, sc, on postoperative days 1 and 2. All animals received the vehicle for delivery of CsA, i.e. 1 ml of olive oil, po, daily, on preoperative days 4, 3, 2 and 1 and on postoperative day 1. All animals received vehicle A, ip, once, intraoperatively and vehicle B, ip, via a mini-pump, for postoperative days 1 and 2. Depending on group assignment, vehicles A and B were used for delivery of SC-19220, a PGE_2 receptor antagonist of the EP_1 subtype [40, 41].

Animals were assigned to 5 groups (Table 1). Each group was comprised of 8 animals.

Groups I and II underwent sham operations. Groups III, IV and V underwent partial hepatectomy. Groups II, IV and V received 20 mg/kg CsA, po, daily, on preoperative days 4, 3, 2 and 1 and postoperative day 1. Group V received 5 mg SCI9220, ip, once, intraoperatively and 20 mg SC-19220, ip, via a mini-pump, for postoperative days 1 and 2. All 40 animals underwent total hepatectomy on postoperative day 2 and their livers were subjected to ex-vivo perfusion.

All animals (n = 40) received BrdUrd and vehicles for the delivery of CsA (oil) and SCI9220 (vehicles A and B). Vehicle B ± SCI9220 was administered via an intraperitoneal mini-pump with delivery equilibrium 4

Table 1. Experimental group assignment.

(n = 8)	**Group I (n = 8)**	**Group II (n = 8)**	**Group III (n = 8)**	**Group IV (n = 8)**	**Group V**
Hepatectomy		√	√	√	√
SC19220					√
CsA				√	√

hours after implantation and a life span of at least 48 hours. Vehicle A ± SC19220 was used to compensate for the mini-pump's 4-hour equilibrating time.

2.4 Surgical Procedures

All animals were operated under light ether anesthesia. Sham operations consisted of laparotomy and mobilization of the liver. Partial hepatectomy included removal of the left lateral and median lobes of the liver (68% hepatectomy) as described by Higgins and Anderson [2], The osmotic mini-pump Alzet model 2ML1 (Alza, Palo Alto, CA, USA) containing vehicle B ± SC19220 was implanted intraperitonealy as described elsewhere [42] at the end of either the sham operation or the hepatectomy. Liver explantation included opening the previous surgical incision, followed by injection of 1500 units of heparin into the spleen. The portal vein was subsequently dissected and a glass pipette (OD 1.5 mm) inserted and secured in place. The animals were exsanguinated to death. Finally, the remaining hepatic attachments were divided and the liver removed.

2.5 Liver Perfusion, Hepatocyte Preparation and Sample Preparation for Flow Cytometric Analysis

Isolated rat livers were perfused as described by Graf and Peterlik [43]. The perfusion was performed through the portal vein with a roller pump TN400 (Ergo Medical, Athens, Greece) at a rate of 3.5 ml/gr/min. Modified Krebs-Henseleit bicarbonate buffer solution was used for the first 30 minutes of perfusion followed by 10 minutes of modified HBSS. Both perfusates were kept at 37 °C with continuous oxygenation. The portal vein pressure varied between 8 and 14 cm H_20. Finally, the liver was perfused for another 10 minutes with 100 ml of re-circulating modified HBSS that contained 15 mg of $CaCl_2$ and 80 mg of type IV collagenase. The lysed liver was washed with Leibowitz medium and filtered through a nylon mesh. Hepatocytes were isolated with three differential centrifugations at 300 rpm and then re-suspended with PBS. The hepatocyte population was 98% pure as calculated by light microscopy after hematoxylin-eosin staining. The viability of the isolated hepatocytes was estimated with the trypan blue exclusion method in a Neubauer hemacytometer [44]. Final re-suspension concentration was 105 cells/ml.

A sample of the purified hepatocytes was stained with the following FITC-conjugated mouse anti-rat monoclonal antibodies: i) OX-18 (anti-MHC class I, monomorphic anti-RTlA[a]). ii) OX-6 (anti-MHC class II, monomorphic anti-RTlB). Another sample of the purified hepatocytes was fixed in 70% methanol for 10 minutes at -20 °C . Following that, formaldehyde (4%) fixation was performed for 10 minutes at room temperature under vortex agitation to prevent cell clumping. The fixed hepatocytes were then treated with acetone (50%) for 10 minutes at -20 °C to achieve cell membrane permeability. Cells were then treated with pepsin (0.2% in 2N HC1) for 60 minutes at room temperature in the dark. After completion of the pepsin treatment, the hepatocytes were washed well with PBS containing 1% OVA 0.5% Tween 20 and re-suspended in 200 µl of PBS with l%OVA and 20µ1 of FITC-conjugated anti-rat monoclonal antibody against BrdUrd.

2.6 Flow Cytometry

Immunofluorescence was quantified by flow cytometry. Cells were analyzed in a FACStar Plus flow cytometer (Becton-Dickinson, Mountain View, CA, USA) equipped with an argon LASER (488 nm). Debris and damaged cells were excluded by gating on a forward and side scatter light properties of hepatocytes. FITC fluorescence was detected in the FL1 channel and stored using logarithmic amplification. Expression of MHC antigens and Brdu staining was determined as the mean fluorescence intensity after substraction of the mean florescence intensity of isotype control value and percent of positive cells (labeling index) . Dotted lines were set on the control antibody (ie. isotype matched control or non-specific labeled Ab) to account for non-specific background. The fluorescent cells (right to the dotted line) are those stained with the FITC-labeled antibodies. Data were evaluated by the FACStar Plus software (Becton-Dickinson, Mountain View, CA, USA).

2.7 Light Microscopy Observations and Hepatocyte Proliferative Activity

Small liver blocks from the caudate lobe were obtained at the time of death. They were fixed by immersion in a solution of 50% alcoholic formalin and 50% modified Krebs-Henseleit bicarbonate buffer solution. They were then paraffin embedded. Following that, 4 mm-thick sections were cut and stained with hematoxylin-eosin. Hepatocyte proliferative activity was estimated by counting the mitosing hepatocytes in 50 consecutive high power fields (HPF: 400X). Cells in prophase before the dissolution of nuclear membranes and late telophase were excluded.

2.8 Statistics

A commercial statistical package was utilized (SPSS 11.0.3, SPSS Inc., Chicago, IL, USA). Continuous variables were summarized using mean and 95% confidence intervals. Statistical significance between groups for continuous variables was evaluated by unpaired Student's *t*-test. All *p*-values are reported.

3. Results

3.1 DNA Synthesis

CsA increased DNA synthesis by rat hepatocytes (Figs. 1 and 2). The mean fluorescence intensity generated by the anti-BrdUrd antibodies was 57.75 (CI: 42.42 - 73.08) for group I, 127.38 (CI: 91.41 - 163.34) for group II, 338.25 (CI: 290.63 - 385.87) for group III, 413.75 (CI: 366.63 - 460.87) for group IV, and 137.00 (CI: 122.23 - 151.77) for group V. CsA had an augmenting effect on liver regeneration between

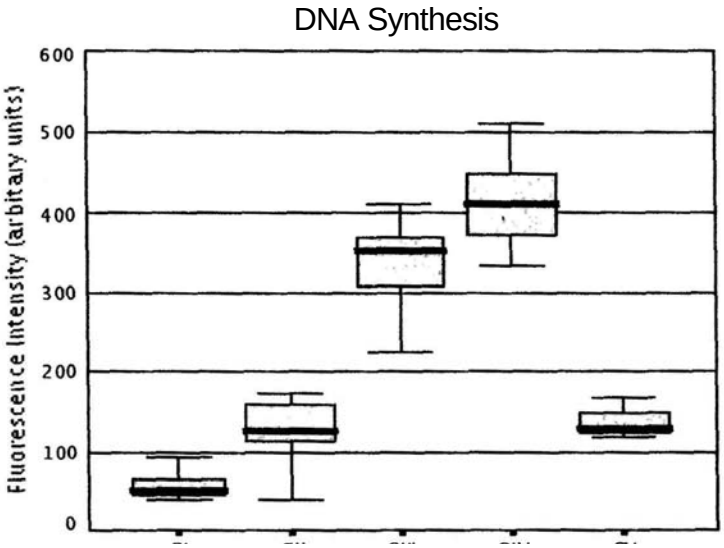

Fig. 1. Quantification of DNA synthesis. BrdUrd was incorporated into the DNA of proliferating hepatocytes. Treatment of the isolated hepatocytes with FITC-conjugated anti-rat monoclonal antibody against BrdUrd led to increased fluorescence emission by dividing cells. Fluorescence was quantified by flow cytometry and depicted in arbitrary units.

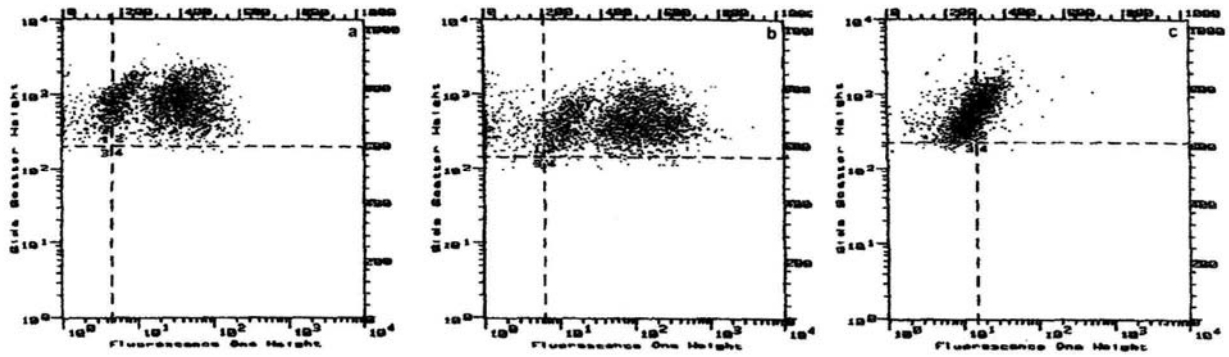

Fig. 2. Dual parameter dot plot of side scatter vs fluorescence of hepatocytes, isolated 48 hours after 2/3 partial hepatectomy and stained with monoclonal antibody anti BrdUrd- FITC : a) group III (PH 2/3), b) group IV (PH 2/3 + CsA), c) group V (PH 2/3 + CsA+ S-19220).

groups III (partial hepatectomy) and IV (partial hepatectomy and CsA) that achieved statistically significance (p = 0.018). Moreover, it was found that CsA increased DNA synthesis in the sham operated animals. Administration of SC-19220 attenuated the CsA augmented proliferating response between groups IV (partial hepatectomy and CsA) and V (partial hepatectomy, CsA and SC-19920) ($p < 0.001$). However, the attenuation was not enough to make the response comparable to that of group I (sham operation), suggesting that the CsA-mediated liver regeneration is partially PGE_2 dependent. Groups II (sham operation, CsA) and V (partial hepatectomy, CsA and SC-19920) emitted similar fluorescence (p — 0.568).

Similar observations among the experimental groups were made when mean DNA synthesis labeling index rather than mean fluorescence intensity was evaluated. Corresponding values for groups I, II, III, IV and V were 6.88% (CI: 5.58 - 8.17), 39.75% (CI: 30.57 - 48.93), 72.75% (60.56 - 84.94), 85.25% (82.10 - 88.4) and 44.38% (35.84 - 52.91), respectively.

3.2 MHC I Expression

Flow cytometry analysis of the isolated normal rat hepatocytes showed moderate grade expression of class I and very low but detectable expression of class II MHC molecules.

Partial hepatectomy increased the expression of hepatocyte MHC class I molecules (Figs. 3 and 5). The mean fluorescence intensity generated by the anti-MHC class I antibodies was 141.88 (CI: 127.41 - 156.34) for group I (sham operation) and 291.13 (CI: 253.18 - 329.07) for group III (partial hepatectomy) ($p < 0.001$). However, the groups that received CsA, with or without SC-19220, did not show increased hepatocyte immunogenicity (MCH I). Indeed, the values of mean fluorescence intensity were 134.63 (CI: 125.61 - 143.64), 167.25 (CI: 139.06 - 195.44) and 180.50 (CI: 156.18 - 204.81) for groups II, IV and V respectively (p = 0.414). This indicates that the CsA-mediated decrease of the hepatocyte immunogenicity (MHC Γ) was not PGE_2-EP_1 receptor dependent.

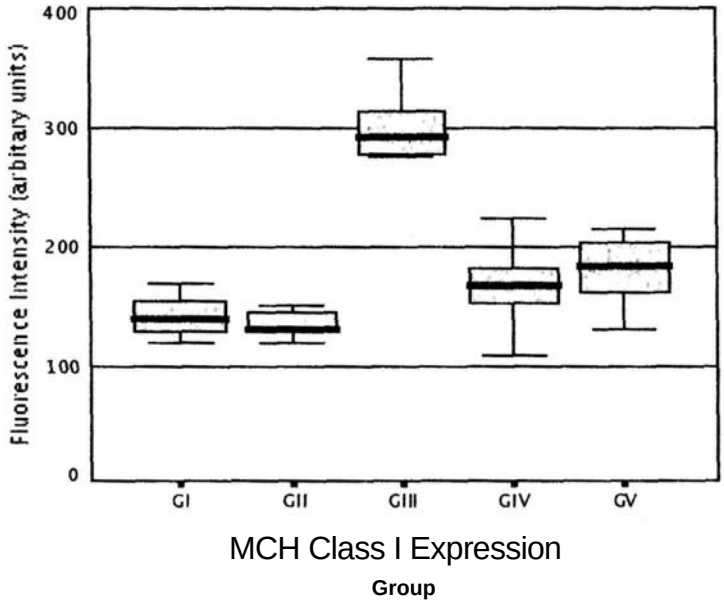

Fig. 3. Quantification of MHC class I expression. Treatment of the isolated hepatocytes with FITC-conjugated mouse anti-rat monoclonal antibodies against MHC class I molecules (OX-18, monomorphic anti-RT1A[a]) leads to increased fluorescence emission by the most immunogenic cells. Fluorescence was quantified by flow cytometry and depicted in arbitrary units.

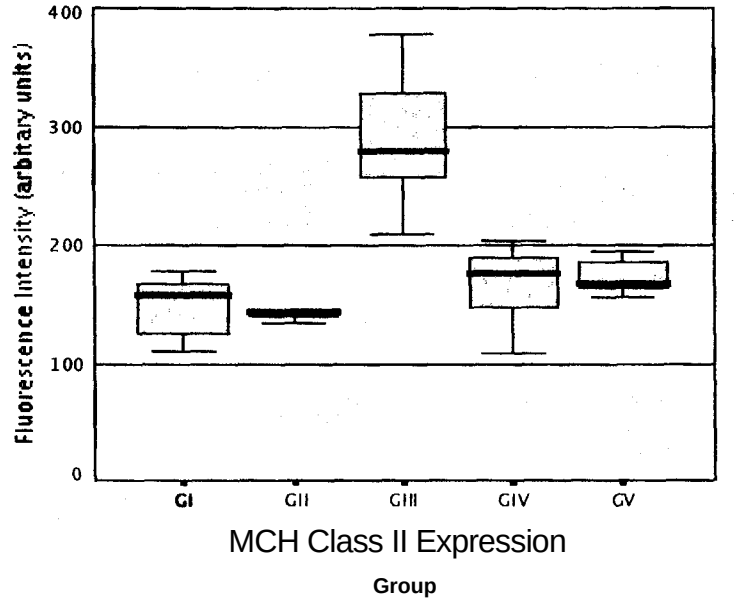

Fig. 4. Quantification of MHC class II expression. Treatment of the isolated hepatocytes with FITC-conjugated mouse anti-rat monoclonal antibodies against MHC class II molecules (OX-6, monomorphic anti-RT1B) led to increased fluorescence emission by the most immunogenic cells. Fluorescence was quantified by flow cytometry and depicted in arbitrary units.

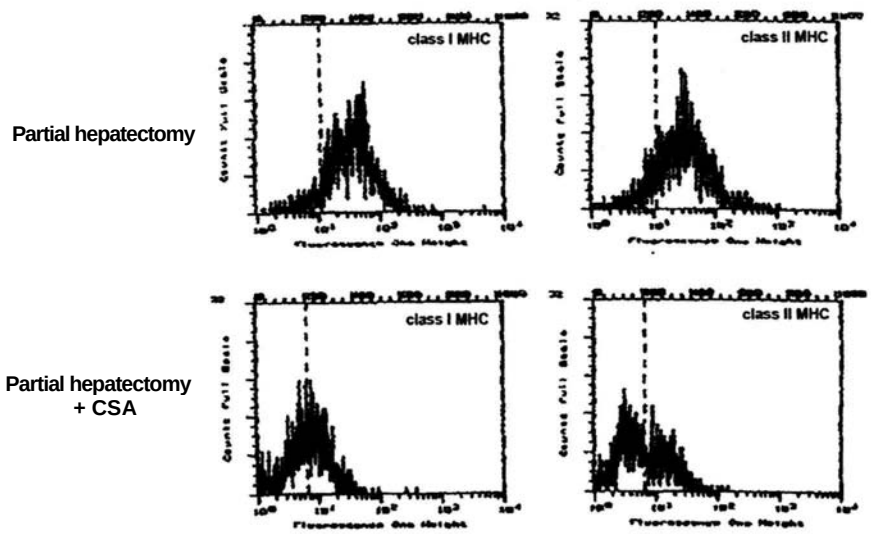

Fig. 5. Surface expression of class I and class II MHC molecules on regenerated hepatocytes (upper panel) and regenerated hepatocytes treated with cyclosporine (lower panel), using monoclonal anti-MHC class I (OX-18, monomorphic anti-RT1A[a]) and anti-MHC class II (OX-6, monomorphic anti-RT1B) antibodies. Histograms show the distribution of antigen expression (fluorescence intensity). Fluorescence intensity is displayed on a log scale versus cell count.

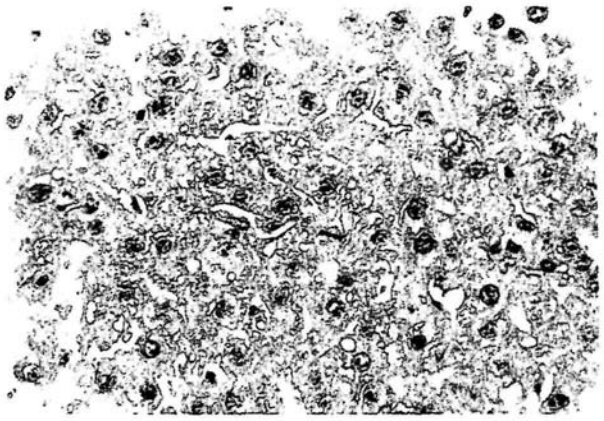

Fig. 6. Hematoxylin-eosin staining of a group III liver specimen (partial hepatectomy). Notice the microvesicular steatosis and the scattered dividing nuclei. High-power field (HPF: 400x).

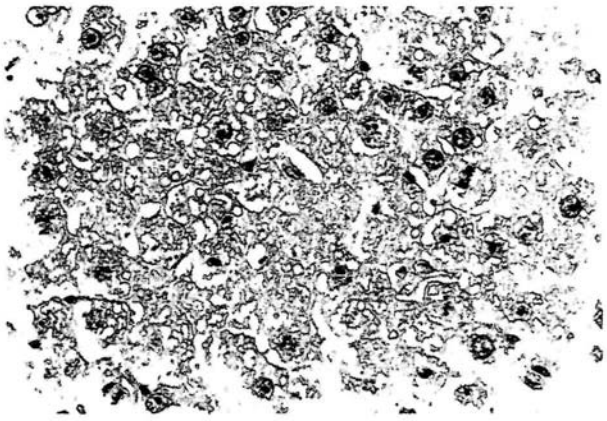

Fig. 7. Hematoxylin-eosin staining of a group IV liver specimen (partial hepatectomy and CsA). Notice the hepatocyte swelling, the bi-columnar hepatocyte sheets, and the increased number of dividing nuclei (7) . High-power field (HPF: 400x).

Similar observations among the experimental groups were made when mean MHC I expression labeling index rather than mean fluorescence intensity was evaluated. The corresponding values for groups I, II, III, IV and V were 60.00% (CI: 50.63 - 69.37), 53.13% (CI: 46.22 - 60.03), 74.63% (66.76 - 82.49), 57.50% (44.84 - 70.16) and 68.25% (63.53 - 72.97), respectively.

3.3 MHC II Expression

Partial hepatectomy increased the expression of hepatocyte MHC class II molecules (Figs. 4 and 5). The mean fluorescence intensity generated by the anti-MHC class II antibodies was 149.13 (CI: 129.04 - 169.21) for group I (sham operation) and 290.88 (CI: 244.90 - 336.85) for group III (partial hepatectomy) ($p < 0.001$). However, the groups that received CsA, with or without the PGE_2 inhibitor, did not show increased hepatocyte immunogenicity (MCH II). Indeed, the values of mean fluorescence intensity were 146.13 (CI: 134.26 - 157.99), 167.25 (CI: 139.58 - 194.92) and 173.25 (CI: 160.55 - 185.95) for groups II, IV and V respectively ($p = 0.288$). This suggests that the CsA-mediated decrease of hepatocyte immunogenicity (MHC II) was not PGE_2 dependent.

Similar observations among the experimental groups were made when mean MHC II expression labeling index rather than mean fluorescence intensity was used for comparison. The corresponding values for groups I, II, III, IV, and V were 47.00% (CI: 38.42 - 55.58), 45.63% (CI: 40.06 - 51.19), 74.75% (65.29 - 84.21), 47.25% (33.04 - 61.46) and 54.63% (47.73 - 61.52), respectively.

3.4 Optical Microscopy

Light microscopy of tissue specimens from groups III (partial hepatectomy) and IV (partial hepatectomy and CsA) revealed characteristic changes of liver regeneration, i.e. hepatocyte swelling, microvesicular steatosis, dividing nuclei, visible nucleoli and bi-columnar hepatocyte sheets. These changes were more prominent in group IV specimens, a fact that verified the augmenting role of CsA in liver regeneration (Figs. 6 and 7). In addition, the number of mitosing hepatocytes per 50 consecutive high-power fields was 118.07 (CI: 109.01 - 127.02) in group III and 148.07 (CI: 139.03 - 157.04) in group IV specimens ($p < 0.001$).

4. Discussion

This study confirmed that CsA augments liver regeneration after partial hepatectomy in rats, a finding that is in consistent with previous reports [5-9]. This was evidenced by both molecular and microscopic findings in our study. Moreover, it was also found that CsA increased DNA synthesis in the sham operated animals. This is supported by the findings of Andres et al. [45] who observed that after 3 hr and 6 hr of incubation with CsA, both the ratio of cells in S phase as well as the levels of cyclins Dl and E increase, but return to normal ranges after 22 hr of incubation. This mitogenic effect is probably transient, since other groups have found an inhibitory or apoptotic effect of CsA on hepatocyte cultures [46, 47].

In the present study, the administration of SC-19220, a PGE_2 receptor antagonist of the EP_1 subtype, attenuated the augmenting action of CsA on DNA synthesis after 2/3 partial hepatectomy in rats. However, the effect was not enough to make the response comparable to that of group I (sham operation), indicating that the CsA-augmentation of liver regeneration is not totally PGE_2 dependent. It has been reported that CsA induces the production of reactive oxygen metabolites in the regenerating liver [48] and through them may activate phospholipase A2, arachidonic acid mobilization and PGE_2 synthesis [49]. The findings of this study suggest that the augmenting action of CsA on liver regeneration is indirect and is mediated, at least partially, through the action of PGE_2 on EP_1 receptor [33, 35].

Flow cytometric analysis of the isolated normal rat hepatocytes in this study showed medium grade expression of class I and very low but detectable expression of class II MHC molecules. Partial hepatectomy significantly induced the expression of class I and class II MHC molecules in regenerating hepatocytes. Previous reports had attributed the increased class II MHC expression to Kupffer cells rather than to hepatocytes [50, 51], a difference that can be explained by the higher sensitivity and specificity of our flow cytometry in relation to their immunohistochemistry [52]. The induction of expression of class I and class II MHC molecules in the regenerating liver can be explained by the increased levels of interferon-γ (IFN-γ), observed after 2/3 partial hepatectomy [53].

The administration of SC - 19220, a PGE_2 receptor EP_1 subtype antagonist, did not have any significant effect on the expression of class I and class II MHC molecules on the hepatocytes of regenerative livers 48 hours after partial 2/3 hepatectomy. This can be explained by previous findings according to which the EP_1 receptor does not have any effect on the expression of class I and class II MHC genes, whereas the inhibitory action of PGE_2 is attributed to the increased intracellular concentration of cAMP via the EP_2 and EP_4 receptors [54, 55]. Consequently, the inhibitory action of SC - 19220 on liver regeneration is due to the inhibition of hepatocyte proliferation rather than to their increased immunogenicity.

The induction of expression of class I and class II MHC molecules by the regenerating hepatocytes 48 hours after partial 2/3 hepatectomy, in conjunction with the previous findings that hepatocytes have the capacity to function as APCs [25, 26], suggests that regenerating hepatocytes participate in the stimulation of the specific immune response during liver regeneration [56]. This enhanced antigen presentation is probably related to the exacerbated acute rejection observed in regenerating small-for-size liver grafts [57, 58]. The experimental protocol of this study does not permit us to clarify whether the regenerating hepatocytes have the capacity to provoke the generation of cytotoxic T lymphocytes, or if they simply induce tolerance. Other experimental models have shown that hepatocytes induce a short-lived cytotoxic response. More specifically, Bertolino et al. [59] found that T cells activated by antigen presentation from hepatocytes express cytotoxic activity for 48 hours, then became anergic, and finally die from apoptosis. In addition, Yoshimura and Kamada [6], have shown that the injection of T lymphocytes from hepatectomized rats four days after hepatectomy reduce regeneration in newly hepatectomized rats. On the contrary, the administration of T lymphocytes isolated from hepatectomized rats ten days after hepatectomy increases the mitotic index in newly hepatectomized rats, a finding that suggests an immunosuppressive effect. More recently, Mêle et al. [60] showed that after allogeneic liver transplantation in normal mice, the increased MHC class II expression is associated with spontaneous regression of rejection. In contrast, the inhibition of MHC class II expression in IFN-γ-deficient mice is associated with accelerated rejection. Moreover, the capacity of the liver to regenerate even after second hepatectomy suggests that there is tolerance to regenerating hepatocytes [61]. According to the above, a hypothetical model could be proposed, in which at the early stages of liver regeneration, hepatocytes would function as antigen presenting cells and would induce the generation of cytotoxic T cells. The temporary cytotoxicity of T cells on regenerating hepatocytes, in conjunction with increased concentrations of other inhibitory factors such as TGF_, would then contribute to the normal inhibitory mechanisms of liver regeneration [18]. Later, upon death by apoptosis of the cytotoxic T cells, tolerance to regenerating hepatocytes would develop.

The administration of CsA significantly reduced the expression of class I and class II MHC molecules by regenerating hepatocytes, 48 hours after partial 2/3 hepatectomy. This can be attributed to: a) a direct inhibitory effect of CsA on the expression of class I and class II MHC genes [21], or b) an indirect effect through the augmentation of PGE_2 synthesis [22]. The present study can not clarify which of the aforementioned mechanisms is responsible for the reduction of MHC expression by CsA.

In conclusion, our findings suggest that the augmentation of liver regeneration by CsA is indirect and can be attributed to: a) a decreased immunogenicity of regenerating hepatocytes due to inhibition of class I and II MHC gene expression 48 hours after partial hepatectomy and b) an increase in liver synthesis of PGE_2 via the EP_1 receptor. Our observations need to be confirmed by further experimentation that will delineate the signal transduction pathways involved. Understanding of the molecular mechanisms that govern CsA-mediated liver regeneration and the immunogenicity of regenerating liver grafts is of paramount importance for the successful clinical application of split and living-related liver transplantation.

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