Transplant Int (1990) 3: 141–145

TRANSPLANT
International

The use of parabiosis for investigating the mechanism of transplantation tolerance in bone marrow chimeras induced by total lymphoid irradiation

A. Eid[1], S. Morecki[2], and S. Slavin[2]

[1] Department of Surgery B and [2] Department of Bone Marrow Transplantation and the Cancer Immunobiology Research Laboratory, Hadassah University Hospital, IL-91120 Jerusalem, Israel

Received December 13, 1989/Received after revision April 27, 1990/Accepted June 8, 1990

Abstract. The mechanism of transplantation tolerance in total lymphoid irradiation (TLI)-induced semiallogeneic bone marrow chimeras without clinical evidence of graft-versus-host disease (GVHD) was investigated using the technique of surgical parabiosis. When held in parabiosis with normal BALB/c mice, BALB/c→ (BALB/c × C57BL/6)F1 (BALB→F1) chimeras survived 7–9 days, significantly ($P < 0.001$) shorter than the 12–19 day survival of normal F1 hybrids kept in parabiosis with normal BALB, and in contrast to indefinite (> 200 days) survival of syngeneic BALB parabiotic partners. When C57 skin grafts were placed on BALB mice held in parabiosis with BALB→F1 chimeras, C57 skin grafts survived 50–60 days, in contrast to 10–14 days in normal BALB recipients ($P < 0.001$). Lethal GVHD, induced in sublethally irradiated F1 recipients by 10^7 BALB spleen cells, could not be delayed or prevented by cotransfer of 10^7 to 30×10^7 tolerant BALB spleen cells obtained from stable BALB→F1 chimeras. GVHD reactivity of BALB spleen cells isolated from BALB→F1 chimeras tolerant of C57 could not be recovered by depletion of Lyt 2 cytotoxic suppressor cells. Taken together, in the absence of suppressive capacity by suppressor cells, these data support functional clonal deletion as the primary mechanism responsible for the maintenance of unresponsiveness to host alloantigens in TLI-induced semiallogeneic chimeras, since no protection against induction of GVHD could be documented in vivo. The transfer of relative unresponsiveness to the host's alloantigens to the normal BALB partner of BALB/BALB→F1 parabiotic pairs following separation is also compatible with the latter conclusion, although transfer of suppressor cells capable of blocking rejection but not GVHD against the same alloantigens cannot yet be formally excluded.

Offprint requests to: S. Slavin

Key words: Total lymphoid irradiation, bone marrow, in mice – Bone marrow, total lymphoid irradiation, in mice – Parabiosis, transplantation tolerance, in mice – Transplantation tolerance, mechanism, in mice

Infusion of allogeneic and semiallogeneic bone marrow (BM) cells following conditioning with total lymphoid irradiation (TLI) was shown to result in alloantigen-specific and permanent transplantation tolerance and stable chimerism without clinical signs of graft-versus-host disease (GVHD) in rodents [5, 14–17, 21]. The tolerogenic effects of TLI were subsequently confirmed in large outbred species, including humans [10, 12, 18]. Although suppressor cell activity has been documented following TLI [22, 23], as well as in well-established chimeras prepared by inoculating allogeneic BM cells into TLI-conditioned mice [13], the cellular basis of this immunological unresponsiveness is still unclear [7–9, 20, 22]. In semiallogeneic chimeras induced by TLI, we could not find conclusive evidence of consistent donor or host-type alloantigen-specific suppression to explain the specific unresponsiveness of the graft versus host [9].

In contrast, we have documented evidence in favor of functional clonal reduction in long-term chimeras with low frequency of cytotoxic T-lymphocyte precursor (CTLp) cells [8, 9, 20]. Interestingly, clonally depleted tolerant donor cells obtained from stable chimeras were capable of clonal expansion with increased CTLp following adoptive transfer into lethally irradiated recipients of donor (syngeneic) MHC, suggesting that the continuous presence of the tolerogenic host-type alloantigens is mandatory for the maintenance of the unresponsive state [9, 20]. In the present study, using the technique of parabiosis, we have designed an experimental model to discriminate active cellular suppression from clonal deletion as the cel-

Table 1. Evidence for chimerism in (BALB/c × C57BL/6) F1 recipients conditioned with TLI prior to infusion of BALB/c bone marrow cells

Experimental group[a]	Cytotoxicity	
	BALB/c anti-C57BL/6 antisera + complement	C57BL/6 anti-BALB/c antisera + complement
Normal BALB/c	< 5%	> 95%
Normal C57BL/6	> 98%	< 5%
Normal (BALB/c × C57BL/6) F1	> 95%	> 95%
BALB/c→(BALB/c × C57BL/6) F1 chimeras prepared with TLI	0%–15%	90%–100%

[a] A minimum of 10 animals were tested in each group

lular mechanism of transplantation tolerance in the semiallogeneic chimeras induced by TLI.

Materials and methods

Mice

Inbred 3–5-month-old BALB/c ($H\text{-}2^d$) (BALB), 6–8-week-old C57BL/6 ($H\text{-}2^b$) (C57), and 3–5-month-old (BALB/c × C57BL/6) F1 ($H\text{-}2^{d/b}$) (F1) mice were purchased from the Hebrew University Animal Farm in Jerusalem, Israel. All mice were kept in standard, unprotected animal facilities during all of the experiments.

Induction of chimerism

Semiallogeneic BALB→F1 chimeras were established as previously reported [9]. Briefly, F1 mice were subjected to TLI consisting of eight daily fractions (×5/week) of 200 cGy (total cumulative dose of 1600 cGy) followed by 200 cGy whole body irradiation (WBI) administered 24 h after the last fraction of TLI. A total dose of 30×10^6 live nucleated BM cells derived from 6–8-week-old BALB donors were injected into the lateral tail vein of F1 recipients 24 h after WBI.

Parabiosis

Parabiosis was established between BALB mice and F1 (BALB/F1), TLI-induced BALB→F1 chimeras (BALB/BALB →F1) and syngeneic BALB mice (BALB/BALB) using an extracelomic technique, as previously described [2]. Briefly, animals were anesthetized with 60 mg/kg sodium pentobarbital intraperitoneally, and a skin incision was made along the opposing dorsolateral sides from the ear to the tail base. The skin edges were dissected free and the abdominal wall, including the peritoneum, was cut from the rib cage to the iliac bone, with care being taken not to injure the gut or the diaphragm. The resulting four muscular edges were resutured using 4-0 continuous prolene. The opposing muscles of the arm, chest wall, and gluteus were sutured using interrupted 4-0 prolene. The skin edges were sutured using 4-0 continuous prolene.

Parabiotic union was assessed radiographically using 0.5 ml Hypaque intravenously. Demonstration of contrast material in the urinary bladder of the noninjected partner was an absolute requirement of true parabiosis. Preliminary experimental work has shown that true parabiotic union occurs 3–4 days after the surgical procedure.

Parabiotic partners were housed individually; their food pellets were placed on the cage floor and the water bottle down low. They were followed closely and immediately separated as soon as one partner died.

Skin graft (SG)

A full-thickness SG from C57 donor mice was placed on the dorsum of BALB partners 3 days following parabiosis as previously described [1]. The SG was considered rejected when most of the skin area became necrotic or turned into black eschar.

Assay for chimerism

The percentage of C57-type cells in skin-grafted BALB mice was assayed using BALB anti-C57 and C57 anti-BALB antisera using a complement (rabbit) dependent microcytotoxicity test, as previously described [9].

Assay for suppressor cells

Groups of 6–10 C57 or F1 mice were exposed to sublethal WBI of 650 cGy. Mice received an intravenous challenge of GVHD consisting of 10^7 BALB spleen cells. Controls received allogeneic BALB spleen alone or an equivalent or three times the amount of BALB cells tolerant of C57 obtained from a stable BALB→F1 chimera. Experimental C57 or F1 mice received a similar inoculum of 10^7 normal BALB spleen mixed with 10^7 or 3×10^7 spleen cells obtained from BALB→F1 chimera in an attempt to investigate whether spleen cells obtained from tolerant chimeras are able to block GVHD in vivo. Clinical signs of GVHD and survival were followed in all groups. Lyt2-positive cells were depleted using monoclonal rat anti-mouse Lyt2 antibodies (kindly provided by Dr. Herman Waldmann, Cambridge University, UK) and rabbit complement.

Statistics

All values are expressed as the mean ± standard deviation. Differences between the various experimental groups were assessed by Student's *t*-test.

Results

Induction of stable chimerism following TLI

All F1 recipients conditioned with TLI were proven to be stable chimeras, as indicated by documentation of ≥ 85% donor type (BALB) cells, using specific C57 anti-BALB and BALB anti-C57 antisera (Table 1). Chimeras showed no evidence of GVHD and all BALB→F1 chimeras prepared in parallel with chimeras used for the present experiments survived longer than 200 days.

Survival of parabiotic partners

BALB mice of syngeneic parabiotic pairs survived indefinitely (> 200 days). F1 hybrids of semiallogeneic parabiotic pairs (BALB/F1) had a mean survival of 15.6 ± 2.5 days (range 12–19 days), while BALB/BALB

Table 2. Survival of BALB/c mice parabiotically attached to either normal or (BALB/c × C57BL/6)F1 (F1) or stable BALB/c→F1 bone marrow chimeras prepared with total lymphoid irradiation

Experimental group	Parabiotic partner	Number of parabiotic pairs	Survival Mean ± SD (days)	P
1	Untreated BALB	10	> 200	
2	Untreated F1	10	15.6 ± 2.5	2 vs 1 < 0.0001
3	BALB→F1 chimeras	10	7.5 ± 0.8	2 vs 3 < 0.001

Table 3. Survival of C57BL/6 skin grafts placed on BALB/c recipients following separation from BALB/BALB→F1 parabiotic partners as compared with normal or sensitized BALB controls. SG, Skin graft

Experimental group	Recipients of C57 SG	Number of animals	C57 SG survival Mean ± SD (days)	P
1	Normal BALB	10	12.0 ± 1.5	
2	BALB sensitized with F1 blood[a]	10	8.0 ± 1.0	2 vs 1 0.05
3	BALB partner of BALB/BALB→F1 parabiotic pairs	10	53.6 ± 2.8	3 vs 1 0.001

[a] BALB/c mice were sensitized by two injections of 0.5 ml F1 blood, 21 and 14 days prior to SG

Table 4. An attempt to document suppressor cells in TLI-induced chimeras by adoptive transfer experiments designed to prevent graft-versus-host disease (GVHD)

Recipients	Number of mice	GVHD challenge by BALB spleen cells	Spleen cells from BALB→F1 chimeras	Median survival (days)
C57	10	0	0	> 100
F1	10	0	0	> 100
C57	10	10^7	0	9
F1	10	10^7	0	27
C57	8	0	10^7	> 100
F1	8	0	10^7	> 100
C57	6	0	3×10^7	> 100
F1	6	0	3×10^7	> 100
C57	6	10^7	10^7	12
F1	6	10^7	10^7	30
C57	6	10^7	3×10^7	14
F1	6	10^7	3×10^7	30
C57	6	0	3×10^7 Lyt2-depleted	> 100
C57	6	3×10^7 Lyt2-depleted	0	14

→F1 chimera parabiotic pairs had a mean survival of 7.5 ± 0.8 days (range 7–9 days; $P < 0.001$; Table 2). Therefore, BALB→F1 chimeras survived significantly shorter than normal F1 mice when they were parabiosed with normal BALB mice, due to GVHD. The normal BALB partners (separated after death of the parabiotic partner) showed no evidence of disease.

Survival of C57 skin grafts on BALB recipients separated from parabiotic partners

C57 SG placed on BALB mice separated from BALB/BALB→F1 chimeras parabiotic pairs survived significantly longer (53.6 ± 2.8 days) than in normal BALB or a control group of BALB mice sensitized with F1 blood transfusions (Table 3). No evidence of chimerism could be demonstrated in BALB mice of either group.

Adoptive transfer experiments to identify potential suppressor cells of GVHD

In order to identify the presence of suppressor cells capable of blocking GVHD reactivity in stable chimeras, sublethally irradiated C57 or F1 mice received 10^7 allogeneic BALB spleen cells obtained from normal mice or from stable chimeras (fewer than 10^7 spleen cells did not cause lethal GVHD in F1 recipients; data not shown). As can be seen in Table 4, all recipients of allogeneic spleen cells obtained from normal donors died of GVHD. BALB spleen cells (10^7) obtained from stable BALB→F1 chimeras caused no GVHD in C57 or F1 recipients, even when the inoculum was increased to 3×10^7. Adoptive transfer of 10^7 or even an excess of 3×10^7 spleen cells obtained from BALB→F1 chimeras failed to delay or prevent GVHD mortality induced by 10^7 normal BALB spleen cells (Table 4).

In order to investigate whether unresponsiveness of BALB cells isolated from chimeras was maintained by Lyt2-positive suppressor cells, spleen cells treated with anti-Lyt2 and rabbit complement were injected into six sublethally irradiated C57 recipients (3×10^7 each) and none died of GVHD. A similar depletion of Lyt2-positive cells of normal BALB spleen cells did not eliminate GVHD capacity in vivo (Table 4). The data provides no evidence that unresponsiveness to C57 was maintained by Lyt2 suppressor cells (Table 4).

Discussion

The absence of overt GVHD in TLI-induced chimeras remains an enigmatic immunological phenomenon. We have previously reported data obtained in vivo and in vitro in support of final clonal reduction as the most likely operative mechanism responsible for the maintenance of specific unresponsiveness to host-type (F1) alloantigens in BALB→F1 as well as C57→F1 bone marrow chimeras [9, 20]. More recently, we have demonstrated the importance of the continuous presence of host-type alloantigens to maintain a previously established tolerance [9]. Nevertheless, the mechanism for the induction of unresponsiveness immediately following TLI is not yet fully explained. In view of the fact that potent suppressor cells were documented following TLI [7, 13, 22, 23], it is

tempting to hypothesize that active cell-mediated suppression may play a role in the initial phase of induction of unresponsiveness by blocking clonal expansion of potentially alloreacting cells, thus leading to a final clonal reduction. However, data presented in this paper does not provide any support for the continuous presence of classical Lyt2 suppressor cells as the mechanism for maintaining unresponsiveness to alloantigens across MHC (Table 4). In the present study, we have further investigated the putative in vivo role of suppressor cells that might be present in well-established tolerance prepared by TLI chimeras using the technique of surgical parabiosis.

Parabiosis represents a unique experimental model for investigation of the role of circulating cells in vivo in which one complete animal is grafted into another; therefore, both host-versus-graft and graft-versus-host reactions could be studied simultaneously. The outcome of parabiotic union is dependent primarily on the MHC disparity between partners. Unilateral MHC antigenic disparity, as in parental and F1 pairs, results in the death of F1 hybrids from GVHD on days 14–21 following parabiosis [18]. In our experiments, too, F1 partners of BALB/F1 hybrid pairs died of GVHD on days 12–19, in agreement with previous observations [18]. In contrast F1 partners of BALB/BALB→F1 bone marrow chimeras prepared with TLI conditioning died on days 7–9 following parabiosis, significantly earlier than normal F1 partners (Table 2). This observation strongly suggests that either active suppression of anti-host alloreactive cells may not be present or that antigen-specific suppressor cells may not play a major in vivo role in maintaining stable chimeras. This observation suggests that clonal reduction is the most likely mechanism responsible for maintenance of unresponsiveness of BALB cells obtained from tolerant BALB→F1 chimeras to the F1 recipients in TLI-induced semiallogeneic chimeras. If tolerance of GVHD were maintained by active cellular suppression of alloreactive clones, it would be expected that partners of BALB→F1 chimeras would survive longer or at least not shorter than normal F1. Experiments presented in Table 4 indicate that even in a situation of a threefold excess of spleen cells obtained from stable chimeras, GVHD could not be blocked, suggesting no role or a minor role for active suppression of alloreactive clones in vivo, although not excluding the possibility that suppressor cells might block or prevent clonal expansion of yet uncommitted T lymphocytes in the ontogeny of alloreactive T cells. It should be borne in mind that when assaying for GVHD suppression in the present experiments, alloantigen-committed mature spleen cells were used as a challenge, whereas in vivo, only newly developing T-cell repertoire may be negatively affected by suppressor cells. Therefore, whenever the mechanism of unresponsiveness is being investigated, one should carefully distinguish between mechanisms of induction and mechanisms for the maintenance of tolerance. In the setting of the present investigations, we suggest that tolerance may not be explained by active suppressor cells operating side by side with alloreactive T lymphocytes.

The intriguing question was why BALB→F1 chimera partners were more susceptible to GVHD than normal F1 (Table 2). There are three possible explanations. The first assumes an ongoing, mild, subclinical GVHD in BALB →F1 chimeras that finally balances out to operational tolerance in stable chimeras that might be aggravated following parabiosis with the influx of large proportions of immunocompetent T lymphocytes of BALB origin. The second explanation is based on our assumption, previously supported by experimental data [9, 20], that unresponsiveness in BALB→F1 chimeras prepared with TLI is based on clonal reduction/deletion. Hence, a constant supply of alloreactive T lymphocytes from the normal BALB partner restores the missing clones, resulting in GVHD as expected. The third possible explanation may be due to the fact that hybrid resistance to parental hematopoietic engraftment in untreated F1 partners may convey to them relative resistance against a parental partner in contrast to BALB→F1 chimeras that have lost their hybrid resistance, being, therefore, immunologically hyporesponsive and more susceptible to a challenge of alloreactive cells [4].

Parabiosis between mice with weak MHC antigenic disparity has been reported to induce tolerance of the parental strain to F1 alloantigens [6]. In our study, C57 skin graft survival was markedly prolonged by BALB mice held in parabiosis with BALB→F1 chimeras, although none of the recipients showed permanent tolerance (Table 2). The exact cellular mechanism mediating this delayed or attenuated BALB anti-C57 alloreactivity is not known. It is tempting to assume that BALB anti-C57 suppressor cells of BALB origin may be transferred from the chimeras to BALB mice after parabiosis. However, if that were the case, BALB anti-C57 GVHD reactivity mediated by the normal BALB partner in F1 recipients loaded with such putative "suppressor cells" should have also been attenuated. Blocking serum factors are also an unlikely explanation for the same reason. In addition, we have previously failed to document blocking serum factors in stable TLI-induced bone marrow chimeras [19]. The possibility that suppressor cells may be able to block alloreactivity of rejection (BALB against C57 skin graft) but not necessarily GVHD in the same direction cannot be excluded but, in our opinion, is unlikely. The alternative explanation, which we favor, is that BALB partners were relatively depleted of anti-C57 alloreactive cells by in vivo allodepletion (homing) to the histoincompatible F1 targets immediately following the parabiosis, as previously shown by other investigators documenting depletion of alloreactive cells by in vivo filtration across MHC barriers [3, 11]. Clonally deleted BALB anti-C57 cells remaining in the BALB separated out from the F1 parabiotic partner of the BALB/BALB→F1 pair and clonally depleted BALB cells derived from the tolerant BALB→F1 chimera should not be able to reject acutely C57 skin allograft and, hence, the prolonged C57 survival in BALB recipients separated out from BALB/BALB→F1 parabiotic pairs. Restoration of alloreactivity (loss of tolerance) by tolerant cells "parked" in a syngeneic host is expected, due to a lack of tolerizing alloantigens in the adoptive recipients of syngeneic toler-

ant cells in view of our previous experimental data [9]. We have previously documented that BALB spleen cells tolerant of C57 alloantigens derived from BALB→F1 chimeras induced by TLI with very low frequency of CTLp cells increased the frequency of CTLp as early as 5 days following "parking" in supralethally irradiated syngeneic (BALB) hosts while the same cells maintained low CTLp following "parking" in tolerogenic (C57) hosts [9]. Whether reparation of alloreactivity in the absence of tolerizing alloantigens is due to de novo clonal expansion, loss of soluble blocking MHC determinants on cell surface alloantigenic receptors, or even a more complex loss of cell-mediated negative feedback control is presently unknown.

In conclusion, surgical parabiosis has been shown to be an interesting in vivo model for addressing issues related to cell-mediated suppression and clonal deletion in transplantation tolerance. Data presented in this study favor clonal reduction as the primary mechanism operating in the maintenance of unresponsiveness in TLI-induced chimeras.

Acknowledgements. The authors wish to acknowledge financial support from the Israel Cancer Association (Career Development Award to S. Slavin) and the United States-Israel Binational Science Foundation (research grant 88-00257/2) that enabled us to carry out this study.

References

1. Billingham RE, Medawar PB (1951) The technique of skin grafting in mammals. J Exp Biol 28: 385
2. Eichwald EJ, Lusgraaf EC, Strainer M (1959) Genetic factors of parabiosis. J Natl Cancer Inst 23: 1193
3. Ford WL, Atkins RC (1971) Specific unresponsiveness of recirculating lymphocytes after exposure to histocompatibility antigen in F1 hybrid rats. Nature 234: 178
4. Knobloch C, Denner TG (1988) Loss of F1 hybrid resistance to bone marrow grafts after injection of parenteral lymphocytes: demonstration of parenteral anti-F1 T killer cells and general immunosuppression in the host. Transplantation 45: 175
5. Leshem B, Tsuberi B, Lebendiker Z, Anafi-Ayalon M, Shalit M, Weiss L, Slavin S, Kedar E (1987) Bone marrow transplantation with T-cell depleted grafts: 2. Reconstitution of immuno-hemopoietic functions in lethally-irradiated mice transplanted with unseparated or T-cell depleted allogeneic bone marrow grafts. Transplantation 43: 814–817
6. Martinez CF, Shapiro LT, Kelman T, Onsted T, Good RA (1960) Tolerance of F1 hybrid skin homografts in parent strain induced by parabiosis. Proc Soc Exp Biol Med 103: 266
7. Morecki S, Weigensberg M, Slavin S (1984) Lectin separation of nonlymphoid suppressor cells induced by total lymphoid irradiation. Eur J Immunol 15: 138–148
8. Morecki S, Leshem B, Weigensberg M, Bar S, Slavin S (1985) Functional clonal deletion versus active suppression in transplantation tolerance induced by total lymphoid irradiation (TLI). Transplantation 40: 201–210
9. Morecki S, Leshem B, Eid A, Slavin S (1987) The role of alloantigen persistence in induction and maintenance of transplantation tolerance. J Exp Med 165: 1468–1480
10. Myburgh JA, Smit JA, Meyers AM, Botha JR, Browde S, Thomson PD (1986) Total lymphoid irradiation in renal transplantation. World J Surg 10: 369–380
11. Roser B, Dorsch S (1979) The cellular basis of transplantation tolerance in the rat. Immunol Rev 46: 55
12. Saper V, Chow D, Engleman ED, Hoppe RT, Levin B, Collins G, Strober S (1988) Clinical and immunological studies of cadaveric renal transplant recipients given total lymphoid irradiation and maintained on low-dose prednisone. Transplantation 45: 540–546
13. Slavin S, Strober S (1979) Induction of allograft tolerance after total lymphoid irradiation (TLI): development of suppressor cells of the mixed leukocyte reaction MLR. J Immunol 123: 942–946
14. Slavin S, Strober S, Fuks Z, Kaplan HS (1976) Long-term survival of skin allografts in mice treated with fractionated total lymphoid irradiation. Science 193: 1252–1254
15. Slavin S, Strober S, Fuks Z, Kaplan HS (1977) Induction of specific tissue transplantation tolerance using fractionated total lymphoid irradiation in adult mice: long-term survival of allogeneic bone marrow and skin grafts. J Exp Med 146: 34–48
16. Slavin S, Fuks Z, Kaplan HS, Strober S (1978) Transplantation of allogeneic bone marrow without graft vs host disease using total lymphoid irradiation. J Exp Med 147: 963–972
17. Slavin S, Reitz B, Bieber CP, Kaplan HS, Strober S (1978) Transplantation tolerance in adult rats using total lymphoid irradiation (TLI): permanent survival of skin, heart and marrow allografts. J Exp Med 147: 700–707
18. Slavin S, Gottlieb M, Bieber C, Hoppe R, Kaplan HS, Strober S, Grumet FC (1979) Transplantation of bone marrow in outbred dogs without graft-versus-host disease using total lymphoid irradiation (TLI). Transplantation 27: 139–142
19. Slavin S, Weiss L, Morecki S, Weigensberg M, Fuks Z (1984) Immunosuppression and induction of transplantation tolerance by fractionated total lymphoid irradiation. In: Slavin S (ed) Tolerance in bone marrow and organ transplantation. Elsevier, Amsterdam, pp 105–152
20. Slavin S, Morecki S, Weigensberg M, Bar S, Weiss L (1986) Functional clonal deletion vs suppressor cell-induced transplantation tolerance in chimeras prepared with a short course of total lymphoid irradiation. Transplantation 41: 680–687
21. Strober S, Slavin S, Gottlieb M, Zan-Bar I, King DP, Hoppe RT, Fuks Z, Grumet FC, Kaplan HS (1979) Allograft tolerance after total lymphoid irradiation (TLI). Immunol Rev 46: 87–112
22. Weigensberg M, Morecki S, Weiss L, Fuks Z, Slavin S (1984) Suppression of cell mediated immune responses following total lymphoid irradiation (TLI). 1. Characterization of suppressor cell of the mixed lymphocyte reaction. J Immunol 132: 971–978
23. Zan-Bar I, Slavin S, Strober S (1978) Induction and mechanism of tolerance to bovine serum albumin after total lymphoid irradiation (TLI). J Immunol 121: 1400–1404