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## Original Contribution

# INTERFERON- $\gamma$ INDUCED EXPRESSION OF MHC ANTIGENS FACILITATES IDENTIFICATION OF DONOR CELLS IN CHIMERIC TRANSPLANT RECIPIENTS

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**Abstract** — After whole organ transplantation, donor bone marrow-derived cells migrate out of the graft into the recipient, leading to establishment of chimerism, which is the first step towards the subsequent induction of donor-specific tolerance. In routine immunohistochemical staining, monoclonal antibodies specific for heterotopic MHC alleles are used to identify donor and recipient cells. However, it is difficult to detect these cells using this technique, in long-term allograft recipients who have a persistently low donor cell population (microchimerism). Because Interferon-gamma (IFN- $\gamma$ ) is known to induce expression of MHC class I and class II cell surface molecules, we used this cytokine 12–48 h before sacrifice, to facilitate the identification of donor and recipient cells in the tissues of animals transplanted with either liver (B10 → C<sup>3</sup>H) or bone marrow (LEW → BN). In long-term allograft recipients, the use of IFN- $\gamma$  for as briefly as 12 h prior to sacrifice, results in marked upregulation of class I and class II antigens, leading to easy identification of ubiquitously distributed low numbers of donor cells.

**Keywords** — Interferon- $\gamma$ ; Chimerism; MHC class I and class II; Rodents

## INTRODUCTION

We have proposed recently that solid organ transplantation leads to a state of mixed allogeneic chimerism with widespread migration of donor passenger leukocytes into the recipient and a reciprocal trafficking of host cells into the transplanted organ (1). In humans, donor-specific class II-positive cells have been identified in the skin, lymph nodes, and blood of HLA mismatched recipients, 27–29 yr after renal transplantation (2), and as long as 23 yr after liver transplantation (3). In rats, under FK506 immunosuppression, donor MHC class

II-positive cells migrate out of the grafted liver into the recipient's lymphoid and nonlymphoid organs (4). Similar observations have also been made in MHC disparate mice, where multiple lineages of donor class I and class II-positive cells were identifiable up to 375 days after liver transplantation (5).

Monoclonal antibodies directed against specific MHC haplotypes are commonly employed to phenotype and characterize donor cells in MHC mismatched chimeric recipients. However, due to dwindling donor cell population and perhaps immunomodulation of donor MHC class I and class II surface molecules, immunohistochemical identification of these cells in long-term allograft recipients becomes very arduous, and the desired result is not always achieved. To facilitate their identification, we have used Interferon-gamma (IFN- $\gamma$ ) to augment the expression of class I and class II antigens.

INF- $\gamma$  is mainly secreted by activated T cells (6) and is known to induce expression of MHC class I antigen on myocytes (7), endothelial and epithelial cells (8,9), lymphocytes (10), fibroblasts (11), and multipotent hematopoietic precursor cell lines (12). It also upregulates the expression of MHC class II molecules on endothelial (12) and epithelial cells (13), on monocytes and macrophages (14,15), and on dendritic cells (16). The expression of class II antigen-associated invariant chain is also upregulated by IFN- $\gamma$  (17). We report here the use of IFN- $\gamma$  to boost MHC class I and class II expression on chimeric donor cells in long-term allograft recipients.

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enue, SC Falk Clinic, University of Pittsburgh, Pittsburgh, PA 15213.

## MATERIAL, METHODS, AND RESULTS

### *In Mice*

Livers from C57Bl/10 donors (B10, H-2k<sup>b</sup>, I-A<sup>b</sup>) were transplanted into C<sup>3</sup>H (H-2k<sup>k</sup>, I-E<sup>k</sup>) recipients by a method described previously (3). Between 1 h and more than 400 days after transplantation each animal in the experimental group was treated intraperitoneally (IP) with  $4 \times 10^5$  U of recombinant IFN- $\gamma$  (rIFN- $\gamma$ , Schering-Plough, Kenilworth, NJ). Animals were sacrificed 48 h after rIFN- $\gamma$  treatment and various lymphoid and nonlymphoid organs harvested. Liver recipients (1 h–400 days > OLTx) without rIFN- $\gamma$  treatment were used as controls. Harvested organs (heart, liver, pancreas, kidney, small bowel, adrenal gland, lungs, tongue, spleen, thymus, cervical, and mesenteric lymph nodes) were embedded in Tissue-Tek<sup>™</sup> (O.C.T Compound, Miles, IN), snap frozen in liquid nitrogen, and sectioned on a Cryostat. The sections were stained with a panel of primary monoclonal antibodies (mAb), the results of which are detailed elsewhere (5). Our paper reports the use of donor-specific anti-class I mAb (mouse IgG<sub>2a</sub>, clone AF6-88.5, PharMingen, San Diego, CA) and anti-class II mAb (mouse IgG<sub>2a</sub>, clone AF6-120.1, PharMingen, San Diego, CA) on recipient's spleens (90 days > OLTx). Isotype-matched irrelevant mAb was used as negative control. Frozen sections were stained by an indirect avidin-biotin immunoperoxidase method as described previously (5). Briefly, air dried sections were fixed in acetone for min, endogenous biotin was blocked with sequential treatment with avidin and biotin blocking reagent (Vector Laboratories, Inc., Burlingame, CA), and then incubated for 45 min with biotinylated primary mouse mAb. Endogenous peroxidase activity was then quenched with 0.6% H<sub>2</sub>O<sub>2</sub>, and finally incubated with streptavidin conjugated peroxidase complex for 30 min (Boehringer Mannheim, Indianapolis, IN). The reaction product was developed with 3-amino-9-ethylcarbazole (AEC), and after counterstaining with hematoxylin, mounted with crystal mount (Biomedica Corp., Foster City, CA). Sections were evaluated for specific cell staining with a Nikon Microphot-FX light microscope.

In spleens of control animals (90 days > OLTx), it was very difficult to identify either donor class I (Fig. 1a) or class II-positive cells (Fig. 1c). Cell staining was trail, and the frequency of weakly stained donor cells was very low. Additionally, donor MHC class I staining was always weaker than class II (Fig. 1a,c). These findings are in accord with our earlier observations in rats, in which we showed that both the number and the intensity of staining of donor cells was low in long-term allograft recipients (4). However, 48 h after rIFN- $\gamma$  treatment brightly stained donor-class I

(Fig. 1b) and class II-positive cells (Fig. 1d) were easily identified in the recipient's spleen. In addition to intensity, the frequency of MHC positive donor cells was also markedly increased. Similar observations were also made in other lymphoid and nonlymphoid organs (data not shown).

### *In Rats*

$2.5 \times 10^8$  bone marrow cells (BMTx) were transplanted from male LEW (RT1l) donors to male Brown Norway (BN, RT1<sup>n</sup>) recipients, which were immunosuppressed with 1 mg/kg/day of FK506 for 14 days, and once a week thereafter for 160 postoperative days. At 12, 24, and 48 h before sacrifice, animals in the experimental group were treated IP with a single dose of  $2 \times 10^7$  U/kg of rIFN- $\gamma$  (Genentech, Inc., San Francisco, CA), and various lymphoid and nonlymphoid organs were harvested and processed as mentioned elsewhere (4). Control animals received BMTx and FK506, without rIFN- $\gamma$ . Skin and cervical lymph nodes were recovered from some experimental animals, prior to rIFN- $\gamma$  treatment, to serve as internal controls. In this study the mAb L-21-6 (mouse anti-rat IgG<sub>1</sub>) was used, which specifically recognizes the invariant chain of the donor MHC class II molecule, but not that of the recipient (18). Isotype-matched irrelevant primary mAb was used as a negative control. Cryosections were stained by indirect immunoperoxidase method using preformed avidin-biotin kit (Vector Laboratories, Inc., Burlington, CA), according to a method described previously (4). The coloration was developed with AEC, counterstained with Harris's hematoxylin and mounted with crystal mount.

Weakly L-21-6-positive donor cells in very low numbers were identified in the spleens of control animals given BMTx under FK506 immunosuppression (Fig. 1e). However, as early as 12 h after rIFN- $\gamma$  treatment, strong class II-positive donor cells were identified at a very high frequency in the recipient's spleen (Fig. 1f). Similar upregulation of class II was also observed 24 and 48 h after rIFN- $\gamma$  treatment in the spleen and other organs of the recipient (data not shown). Furthermore, cervical lymph nodes and skin obtained from the same BMTx recipients before and after rIFN- $\gamma$  treatment showed marked upregulation of donor class II after but not before rIFN- $\gamma$  enhancement (data not shown). Additionally, treatment with supernatant from 3-day MLR cultures, which has IFN- $\gamma$  among other cytokines (19), also leads to marked upregulation of donor class II in the recipient's organs (data not shown).

## CONCLUSIONS

The results of these experiments show that IFN- $\gamma$  can be successfully used to boost the expression of ~~both~~

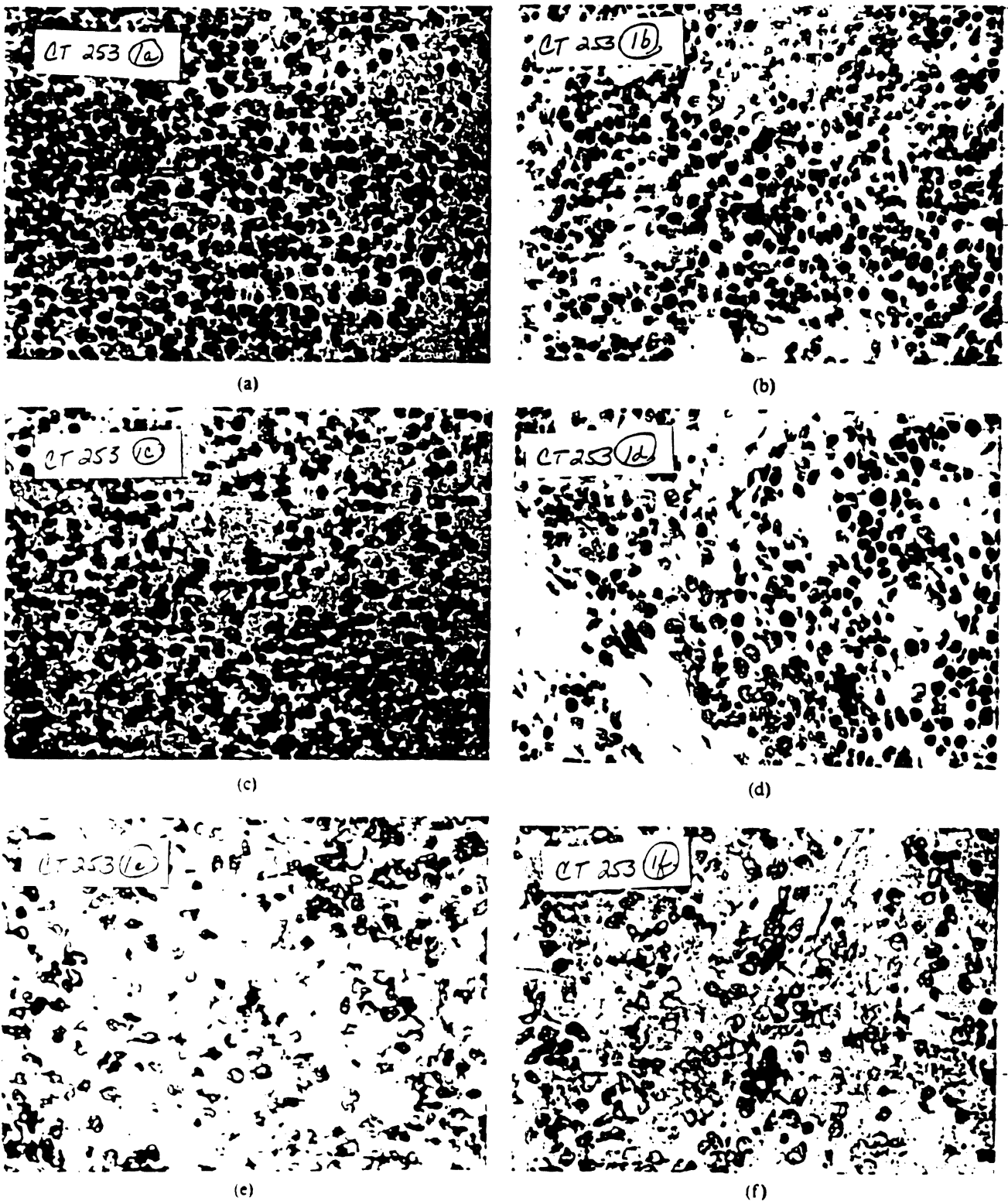


Fig. 1. Indirect immunoperoxidase staining of spleen sections from C<sup>3</sup>H recipient of B10 liver (90 days > OLTx), without (a) and with (b) rIFN- $\gamma$  treatment. The sections were stained with donor-specific anti-class I mAb. Note the intensely stained donor class I-positive cells (f) in (b) but not in (a). Figure 1(c,d): Indirect immunoperoxidase staining of spleen sections from recipient (C<sup>3</sup>H) of B10 liver (90 days > OLTx), incubated with donor-specific anti-class II mAb, without (c) and with (d) rIFN- $\gamma$  treatment. Donor class II-positive cells stained with red reaction product (f) are much more evident in (d) but not in (c). Figure 1(e,f): sections of spleen from BN recipient of LEW bone marrow (160 days > BMTx), without (e) and with (f) rIFN- $\gamma$  treatment. The sections were stained with L-21-6 mAb (which reacts with invariant chain of LEW MHC class II, but not BN). Donor class II-positive cells that show the red reaction product (f) are much brighter and more frequent in (f) than in (e). Sections were stained with indirect immunoperoxidase method. (Original magnification  $\times 260$ ).

MHC class I and class II molecules in chimeric recipients, making it possible for the first time to extensively phenotype and characterize the lineages of these cells in allograft recipients. The question may be asked if IFN- $\gamma$  administration is inherently immunomodulating and therefore an artifact in the system. However, the experimental endpoints are cell localization not their immunologic consequences. Presumably, the cells are in place by the time IFN- $\gamma$  is administered. We therefore believe that IFN- $\gamma$  treatment before sacrificing the animals may allow a much greater delineation of the true magnitude of the so-called microchimerism in a number of experimental models. We see no way in which IFN- $\gamma$  could significantly influence either the extent or constituency of chimerism because rIFN- $\gamma$  treatment for as brief as 12 h can lead to MHC class II upregulation.

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