# THE MURINE CELL SURFACE ANTIGEN PC-1 AS A MARKER FOR PLASMA CELL

*Alida R. Harahap*[*] *and James W.Goding*[**]


## ABSTRACT

The murine plasma cell antigen PC-1 is a cell surface glycoprotein which is disulfic-bond homodimer of Mr 115 kD. It is expressed in large amounts of neoplastic plasma cell and on the majority of haemolytic plaque-forming cells[1]. It is also found in non-lymphoid tissues.[1,2] In this paper a report of detailed distribution of PC-1 in a variety of normal lymphoid tissues using radioiodinated IR-518 monoclonal anti-PC-1 antibody as a probe is given, followed by autoradiography. Because the number of plasma cells in normal condition is very small, mice infected with *Mesocestoides corti* have been used. These *M.corti* infected mice have high levels of IgGI in their serum (Mitchell et al, 1977). Hence, it was expected that the number of plasma cells may also be increased.

Most small lymphocytes are negative of weakly positive. Cells that had morphology of typical plasma cells were mostly positive. The large immunoglobulin-containing cells found in peritoneum of *M.corti* infected mice were strongly positive for PC-1. These results show that PC-1 antigen is expressed on normal plasma cells, the normal counterpart of myeloma cells. Therefore it can be used as a plasma surface marker.


## INTRODUCTION

When resting B lymphocytes differentiate into antibody-secreting cells, the high rate of secretion of antibody by plasma cells must be accompanied by the coordinated switching on of a number of genes. The products of these genes presumably include cells surface receptors, membrane proteins mediating adhesion or homing, and apparatus for packaging and transport of immunoglobulin. It is therefore to be expected that there may be changes at the cell surface. Some of these cell surface molecules can be used as markers for a particular stage of B cells.

Relatively few plasma cell specific antigens have been described. One of these was the PC-1 antigen, first described by Takahashi et al in 1970[1]. This protein has MW of 240 kD (unreduced) and 120 kD (reduced)[2]. It has unusual transmembrane orientation; its carboxy terminus is extracellular and its amino terminus is in the cytoplasm[3].

Expression of PC-1 antigen in the lymphoid cells has been previously shown to accompany the maturation of the cell into the final stage of B cell[1,2,4]. Almost all of these studies have been done using malignant B cell


* Department of CLinical Pathology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

** Department of Pathology and Immunology Monash University, Alfred Hospital, Victoria, Australia

lines. There is evidence for the presence of PC-1 in normal plasma cells: polyclonal allo-antisera in th presence of complement were able to suppress the activity of plaque forming cells from spleen of mice immunized with sheep red blood cells[1]. Immunoprecipitation of cell surface labelled protein from normal spleen cells, followed by SDS-PAGE and autoradiography, also showed that PC-1 is present in normal spleen[2]. However, these methods could not describe the type of cells expressing the PC-1 antigens, and data concerning the morphology of PC-1 positive cells was lacking.

To see whether the PC-1 antigen can be used as specific marker for plasma cells, we conducted a systematic examination of the nature of lymphoid cells bearing PC-1.

## MATERIALS AND METHODS

### Mice

BALB/c and C57B1/6 mice were from the central animal facilities, Monash University, or from stocks maintained at the Walter and Eliza Hall Institute. They were maintained in a conventional environment. All mice used were 8-12 weeks old.
*Mesocestoides/corti*-infected BALB/c and C57B1/6 mice were from Walter and Eliza Hall Institute. These mice were injected intraperitoneally with 25 µl of 70% *M.corti* suspension in phosphate-buffered saline (PBS) and used 2 months later.

### Cells lines

Myeloma cell line NS-1[5] was grown in Dulbecco's modification of Eagle's medium (DMEM, Flow Laboratory, Australia) supplemented with 10% fetal calf serum (FCS, Flow Laboratory, Australia). Pre-B cell line 18.81 was grown in DMEM supplemented with 10% FCS, 0.1 mM asparagine (Sigma, USA), and 0.05 mM 2-mercaptoethanol (Merck, West Germany).

### Antibodies

Monoclonal IgGI antibody IR-518 against the BALB/c allele of PC-1[6] was produced as ascites in BALB/c nude mice. IgGI anti-Leishmania mouse monoclonal antibody, clone WIC 79.3[7], was a gift from Dr. E. Handman (Walter and Eliza Hall Institute).

Both monoclonal antibodies were purified by ion exchange chromatography on DEAE-Sepharose, with linear salt gradient elution, and then iodinated using chloramine T method using 5 ul of 1 mg/ml chloramine T (Sigma, USA) and 100 uCi of $Na^{125}$ I (Amersham, England), usually resulting in an average substitution of about one iodine atom per protein molecule.

### Preparation of Cell Suspension from Lymphoid Organs

Spleen was placed into 10 ml 5% FCS in PBS (=5% FCS), then cut into small pieces with edges of glass slides. Cells were gently squeezed between frosted ends of two microscope slides, then transferred into 10 ml plastic tube, and left on ice for 5-10 min, during which time the debris precipitated. The cells without the debris (top 90% of preparation) were then transferred into a fresh tube, centrifuged at 800g, 4°C, for 5 min, and washed twice in 5% FCS.
Lymph nodes were prepared similarly, but teased using needles.

Peritoneal cells were obtained by lavage of the peritoneal cavity with 10 ml of 5% FCS. Cells were centrifuged and washed as for spleen cells. Peritoneal cells from *M.corti* infected mice were obtained similarly, except

the exudate was left for 5-10 min in 10 ml tube to precipitate the parasite, and the cell suspension without *M.corti* was transferred into a fresh tube.

Nucleated cells in the suspension were counted in an Improved Neubauer haemocytometer using 3% acetic acid as a diluent to lyse erythrocytes. Viability was assessed by mixing equal amounts of cell suspension and acridine orange/ethidium bromide (1 part per million in PBS), and scoring under a fluorescence microscope with filter for fluorescein. Green colour indicates living cells, and orange indicated dead cells.

**Autoradiography of Cell Smears**

All steps were carried out at 4°C. Twenty million cells (with viability more than 95%) were mixed with $10^6$ cpm of iodinated antibody in PBS containing 5% FCS, and held for 30 min on ice. Cells were then washed twice by loading the cells on top of 2 ml 50% FCS and centrifuging at 800g. Cell pellets were resuspended in about 20 µl of 20% FCS. Cell smears were made from these suspensions, allowed to dry at room temperature, and fixed in absolute methanol for 30 min at room temperature.

Dried slides were dipped in Kodak NTB2 photographic emulsion (diluted 1:1 in distilled water) at 42°C in the dark, dried in a metal box with cool air flow for 1-2 hrs, and then transferred into a light-tight plastic box containing *Drierite* dessicant crystals. Exposure was at 4°C for 1-5 days. Slides were developed in D-19 developer (Kodak) for 5 min, rinsed briefly in tap water, and fixed in Ilfospeed 2000. Fixer/Replenisher (Ilford diluted 1:5 with tap water) containing 25% Agfa Aditan hardener for 5 min, and then with running tap water for 5 min. Counter staining was done using 10% Giemsa (BDH Chemicals, England) in tap water for 10-30 min. Slides were mounted with Histomount (National Diagnostic, New Jersey) and covered with cover slips.

Photography was done using a Leitz Wetzlar Orthomat microscope and camera and Kodak Ektachrome 160 reversal film.

**Scoring**

For each smear, 500 cells were scored and classified as lymphocytes plasma cells, granulocytes or macrophages according to their histological characteristics, based on description by Nossal (1959). The lymphocyte has a dark purplish-blue nucleus with dense chromatin pattern, and a scanty, pale blue cytoplasm. The plasma cell has very dense and clumpy chromatin, one or more eccentric nuclei, bulky basophilic cytoplasm, and a perinuclear clear zone corresponding to the Golgi region. The murine granulocyte has an annular nucleus and granular cytoplasm. The eosinophil cytoplasmic granules. The macrophage characteristically has an irregular outline and a relatively small nucleus placed anywhere in the cell.

Lymphocytes were grouped into small (4-6 u nucleus diameter) and large lymphocytes (more than 7 u nuclear diameter) as described by Nossal and Makela[8], except that medium and large lymphocytes were counted as large lympocytes.

The number of grains per cell was counted and the background counts were established at less than 5 grains per cell at 5 days exposure. All grain counts were restricted to grains overlying the cells. A cell was classified as *positive* if it had 5 or more grains at 5 days exposure, and *strongly positive* if it had more than 14 grains at 5 days exposure. An exposure time of 5 days was used unless otherwise mentioned.

## RESULTS

NS-1 myeloma cells incubated with IR-518 monoclonal antibody were very strongly positive. Autoradiogram of the slide after 24 hours exposure showed more than 30 grains per cell. Assuming a roughly linear relationship between grain numbers and exposure times less than one half-life, this would correspond to about 150 grains per cell at 5 days. All other controls gave only a background staining.

The *background* grains between cells was considerably greater when most cells were strongly positive, for example when NS-1 cells were used. This was found to be due to antibody coming of the cells during fixation with absolute methanol.[9] The only solution was using glutaraldehyde as a fixative, but this resulted in poorer staining with Giemsa, and was not done routinely.

### PC-1 Positive Cells in Normal BALB/c Mice

In normal BALB/c mice, the nucleated spleen cells consisted of about 57% small lymphocytes, 40% large lymphocytes, less than 1% plasma cells, less than 1% monocytes, and 1% granulocytes (Table 1). Occasional normoblasts (nucleated red cells) were also seen. These resemble lymphocytes but have a thicker dark blue rim of cytoplasm that is visible around the entire periphery of the nucleus.

In the spleen (Table 2 and Fig. 1a), the great majority (90%) of small lymphocytes were PC-1 negative, 10% were only weakly positive, but none of them had more than 9 grains per cell. About 27% of large lymphocytes were PC-1 positive, 15% of these were strongly positive (15 or more grains per cell). Cells identified morphologically as plasma cells were very rare or not present at all. Macrophages and granulocytes comprise only a very small fraction of all spleen cells, and all were PC-1 negative.

In the lymph node (Table 3 and Fig. 1b), the vast majority of small lymphocytes were PC-1 negative, but occasional cells (3% of small lymphocytes) were weakly positive. None of the small lymphocytes had more then 9 grains per cell. About a quarter of large lymphocytes were positive; of these about 27% had 15 or more grains. Macrophages and granulocytes were either rare or not observed in lymph node.

In the peritoneal washings (Table 4 and Fig. 1c), about a quarter of small lymphocytes were weakly PC-1 positive. None were strongly positive. About half of large lymphocytes were positive, 7% of these had 15 or more grains. Morphological plasma cells were very rare. Only 5 cells were observed, of which 3 wer strongly PC-1 positive.

### PC-1 Positive Cells in M. Corti Infected Mice

It is known that mice infected with *M.corti* have extremely high levels of IgG1 in their serum[10]. As PC-1 is expected to be an antigenic marker for this cell type, it was expected that the levels of PC-1 protein would be increased as compared to uninfected mice. However, van Driel et al[2] observed that the increase in PC-1 mRNA or protein in spleen was only about 2.5 fold, and not commensurate with the observed increase in IgG1 levels.

In the spleen (Table 5 and Fig. 2a), 15% of small lymphocytes were PC-1 positive. Of these, about 16% had 15 or more grains (strongly positive). About one third or large lymphocytes were positive; of these about one third were strongly positive. Surprisingly, no morphological plasma cells were ob-

served in the spleen. Macrophages were rare, and all were PC-1 negative. The number of granulocytes was higher than in uninfected mice. Most of them were eosinophils, and all were PC-1 negative.

In peritoneal washings (Table 6 and Fig. 2b), about halve of cells had an unusual morphology. Many cells were very large, with prominent pale-blue cytoplasm, and no perinuclear clear zone. Their nuclei were eccentric and sometimes multiple. Cell smears were stained for the presence of the cytoplasmic immunoglobulin using FITC conjugated (Fab') fragments of sheep anti mouse Ig (Silenus, Australia). Almost all the large cells had cytoplasmic Ig, as shown by a characteristic granular cytoplasmic fluorescence, indicating that they are plasma cells. Cell smears were incubated with PBS containing 1mg/ml DAB and 0.01% $H_2O_2$ to test for peroxidase activity. The majority of the large cells had no peroxidase activity, suggesting that they were not macrophages. However, latest experiments by Donald Metcalf (WAlter and Eliza Hall Inst.; personal communication) showed that these cells gave positive reaction to several macrophage markers.

About 52% of peritoneal cells from these mice were granulocytes, and of these about 60% were eosinophils, consistent with the parasite infection.

Five days autoradiographs of peritoneal cells from *M.corti*- infected mice showed a very strong reaction with anti-PC-1 antibody, causing a very high background. Because of the strong reactivity of the cells with $^{125}$I-IR518 antibody, it was impossible to see the morphology of the cells and to count the number of PC-1 positive cells. For this reason, exposure time was decreased from 5 days to overnight. Virtually all (97%) of these unusual large cells were PC-1 positive, and of these 94% were strongly positive. Granulocytes were all PC-1 negative.

## DISCUSSION

### Normal mice

The majority of small lymphocytes in normal mice were PC-1 negative or only weakly positive. Small lymphocytes are mostly resting lymphocytes and generally do not secret antibody. On the other hand, large lymphocytes were more often PC-1 positive, and expressed more PC-1 as compared to small lymphocytes. This results are consistent with previous studies which showed that PC-1 is expressed in the antibody secreting plasma cells[1,2]. It seems likely that the expression of PC-1 in lymphoid cells correlates with the activity of the cells. Peritoneal washings had more large lymphocytes and plasma cells than other lymphoid organs. Not only the proportion of PC-1 positive peritoneal cells was higher, but also the positivity was stronger than in the spleen or lymph nodes.

### *M.corti*-infected mice

These mice provided an interesting opportunity to examine *normal* (i.e, non-neoplastic) plasma cells. It is interesting to note that few morphological plasma cells were found in spleen, in spite of high IgG1 levels, and few PC-1 cells were found in spleen. This result is consistent with data of van Driel et al[2], who found only modest increasing in Pc-1 expression in spleens of *M. corti*-infected mice had large numbers of cells that were not clearly *plasma cells*, and they express high level of PC-1 antigen. Further study have to be done to decide the type of these cells. If it is proved that they are mac-

**Table 1. Distribution of cells in the spleen, lymph node and peritoneum of normal, nude, and *M.corti*-infected mice**

| | NORMAL | *M.CORTI* |
|---|---|---|
| SPLEEN | | |
| Small lymphocytes | 57% | 68% |
| Large lymphocytes | 40% | 27% |
| Plasma cells | <1% | <1% |
| Macrophages | 1% | 3% |
| LYMPH NODE | | |
| Small lymphocytes | 54% | ND |
| Large lymphocytes | 45% | ND |
| Plasma cells | <1% | ND |
| Macrophages | <1% | ND |
| Granulocytes | <1% | ND |
| PERITONEAL | | |
| Small lymphocytes | 6% | 2% |
| Large lymphocytes | 76% | 6% |
| Plasma cells | 1% | 39% |
| Macrophages | 16% | <1% |
| Granulocytes | 1% | 52% |

ND, not determined; <1%, less than 1%.

**Table 2. Distribution of PC-1 positive* cells in the spleen of normal BALB/c and C57B1/6 mice**

| CELLS | GRAINS PERCELL | + IR-518 | | + WIC79.3 | |
|---|---|---|---|---|---|
| | | BALB | C57B1 | BALB | C57B1 |
| Small lymphocytes | 0 | 110 | 280 | 260 | 225 |
| | 1 - 4 | 155 | 25 | 18 | 20 |
| | 5 - 9 | 30 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| Large lymphocytes | 0 | 44 | 154 | 177 | 191 |
| | 1 - 4 | 96 | 31 | 33 | 33 |
| | 5 - 9 | 38 | 0 | 0 | 0 |
| | 10 -14 | 7 | 0 | 0 | 0 |
| | 15 or >15 | 8 | 0 | 0 | 0 |
| Plasma cells | 0 | 2 | 0 | 0 | 8 |
| | 1 - 4 | 0 | 0 | 0 | 1 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| Macrophages | 0 | 4 | 1 | 2 | 5 |
| | 1 - 4 | 1 | 0 | 0 | 1 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| Granulocytes | 0 | 4 | 9 | 10 | 14 |
| | 1 - 4 | 1 | 0 | 0 | 2 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -15 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| | TOTAL | 500 | 500 | 500 | 500 |

*, more than 4 grains per cell; + IR-518, reacted with anti PC-1 monoclonal antibody; + WIC 79.3, reacted with anti-*Leishmania* monoclonal antibody; BALB, spleen cells from normal BALB/c mice; C57B1, spleen cells from normal C57B1/6 mice; >15,more than 15.

**Table 3. Distribution ofPC-1positive* cells in the lymph node of normal BALB/c and C57B1/6 mice**

| CELLS | GRAINS PERCELL | + IR-518 | | + WIC79.3 | |
|---|---|---|---|---|---|
| | | BALB | C57B1 | BALB | C57B1 |
| Small lymphocytes | 0 | 96 | 337 | 265 | 403 |
| | 1 - 4 | 162 | 31 | 17 | 21 |
| | 5 - 9 | 7 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| Large lymphocytes | 0 | 49 | 106 | 183 | 65 |
| | 1 - 4 | 129 | 25 | 30 | 10 |
| | 5 - 9 | 31 | 0 | 1 | 0 |
| | 10 -14 | 9 | 0 | 0 | 0 |
| | 15 or >15 | 15 | 0 | 0 | 0 |
| Plasma cells | 0 | 0 | 0 | 1 | 0 |
| | 1 - 4 | 1 | 0 | 0 | 0 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or>15 | 0 | 0 | 0 | 0 |
| Macrophages | 0 | 0 | 0 | 1 | 1 |
| | 1 - 4 | 0 | 0 | 0 | 0 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or>15 | 0 | 0 | 0 | 0 |
| Granulocytes | 0 | 1 | 1 | 1 | 0 |
| | 1 - 4 | 0 | 0 | 1 | 0 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -15 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| | TOTAL | 500 | 500 | 500 | 500 |

*, more than 4 grains per cell; + IR-518, reacted with anti PC-1 monoclonal antibody; + WIC 79.3, reacted with anti-*Leishmania* monoclonal antibody; BALB, lymph node cells from normal BALB/c mice; C57B1, lymph node cells from normal C57B1/6 mice; >15,more than 15.

**Table 4. Distribution of PC-1 positive* cells in the peritoneum of normal BALB/c and C57B1/6 mice**

| CELLS | SGRAINS PERCELL | + IR-518 | | + WIC79.3 | |
|---|---|---|---|---|---|
| | | BALB | C57B1 | BALB | C57B1 |
| Small lymphocytes | 0 | 3 | 89 | 30 | 180 |
| | 1 - 4 | 19 | 3 | 3 | 6 |
| | 5 - 9 | 7 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| Large lymphocytes | 0 | 25 | 266 | 264 | 227 |
| | 1 - 4 | 215 | 50 | 72 | 20 |
| | 5 - 9 | 138 | 1 | 0 | 0 |
| | 10 -14 | 39 | 0 | 0 | 0 |
| | 15 or >15 | 13 | 0 | 0 | 0 |
| Plasma cells | 0 | 0 | 7 | 3 | 4 |
| | 1 - 4 | 2 | 2 | 4 | 3 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 1 | 0 | 0 | 0 |
| | 15 or >15 | 2 | 0 | 0 | 0 |
| Macrophages | 0 | 2 | 29 | 59 | 27 |
| | 1 - 4 | 0 | 20 | 49 | 12 |
| | 5 - 9 | 2 | 1 | 6 | 1 |
| | 10 -14 | 3 | 0 | 0 | 0 |
| | 15 or >15 | 17 | 2 | 0 | 0 |
| Granulocytes | 0 | 8 | 22 | 7 | 15 |
| | 1 - 4 | 4 | 8 | 3 | 5 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -15 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| | TOTAL | 500 | 500 | 500 | 500 |

*, more than 4 grains per cell; + IR-518, reacted with anti PC-1 monoclonal antibody; + WIC 79.3, reacted with anti-*Leishmania* monoclonal antibody; BALB,peritoneal cells from normal BALB/c mice; C57B1, peritoneal cells from normal C57B1/6 mice; > 15,more than 15.

**Table 5. Distribution of PC-1 positive* cells in the spleen of *M.corti*-infected BALB/c and C57B1/6 mice**

| CELLS | GRAINS PERCELL | + IR-518 | | + WIC79.3 | |
|---|---|---|---|---|---|
| | | BALB | C57B1 | BALB | C57B1 |
| Small lymphocytes | 0 | 88 | 393 | 360 | 349 |
| | 1 - 4 | 175 | 36 | 20 | 45 |
| | 5 - 9 | 34 | 0 | 0 | 0 |
| | 10 -14 | 6 | 0 | 0 | 0 |
| | 15 or> 15 | 8 | 0 | 0 | 0 |
| Large lymphocytes | 0 | 28 | 47 | 93 | 75 |
| | 1 - 4 | 77 | 12 | 18 | 21 |
| | 5 - 9 | 31 | 0 | 0 | 0 |
| | 10 -14 | 7 | 0 | 0 | 0 |
| | 15 or >15 | 20 | 0 | 0 | 0 |
| Plasma cells | 0 | 0 | 1 | 1 | 0 |
| | 1 - 4 | 0 | 3 | 0 | 0 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or>15 | 0 | 0 | 0 | 0 |
| Macrophages | 0 | 0 | 0 | 0 | 0 |
| | 1 - 4 | 2 | 1 | 0 | 0 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or> 15 | 0 | 0 | 0 | 0 |
| Granulocytes | 0 | 6 | 7 | 6 | 5 |
| | 1 - 4 | 18 | 0 | 2 | 5 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -15 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| | TOTAL | 500 | 500 | 500 | 500 |

*, more than 4 grains per cell; + IR-518, reacted with anti PC-1 monoclonal antibody; + WIC 79.3, reacted with anti-*Leishmania* monoclonal antibody; BALB, spleen cells from *M.corti*-infected BALB/c mice; C57B1, spleen cells from *M.corti*-infected C57B1/6 mice; > 15,more than 15.

**Table 6. Distribution of PC-1 positive* cells in the peritoneum of *M.corti*-infected BALB/c and C57B1/6 mice**

| CELLS | GRAINS PERCELL | + IR-518 | | + WIC79.3 | |
|---|---|---|---|---|---|
| | | BALB | C57B1 | BALB | C57B1 |
| Small lymphocytes | 0 | 1 | 110 | 15 | 86 |
| | 1 - 4 | 0 | 8 | 1 | 11 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| Large lymphocytes | 0 | 4 | 158 | 27 | 121 |
| | 1 - 4 | 10 | 57 | 4 | 69 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 11 | 0 | 0 | 0 |
| Plasma cells | 0 | 0 | 39 | 56 | 45 |
| | 1 - 4 | 7 | 57 | 90 | 104 |
| | 5 - 9 | 4 | 14 | 3 | 5 |
| | 10 -14 | 9 | 0 | 0 | 0 |
| | 15 or >15 | 224 | 2 | 0 | 0 |
| Macrophages | 0 | 0 | 8 | 0 | 10 |
| | 1 - 4 | 1 | 6 | 2 | 0 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -14 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 2 | 0 | 0 | 0 |
| Granulocytes | 0 | 85 | 28 | 251 | 37 |
| | 1 - 4 | 140 | 13 | 51 | 12 |
| | 5 - 9 | 0 | 0 | 0 | 0 |
| | 10 -15 | 0 | 0 | 0 | 0 |
| | 15 or >15 | 0 | 0 | 0 | 0 |
| | TOTAL | 500 | 500 | 500 | 500 |

*, more than 4 grains per cell; + IR-518, reacted with anti PC-1 monoclonal antibody; + WIC 79.3, reacted with anti-*Leishmania* monoclonal antibody; BALB, spleen cells from *M.corti*-infected BALB/c mice; C57B1, spleen cells from *M.corti*-infected C57B1/6 mice; > 15,more than 15.

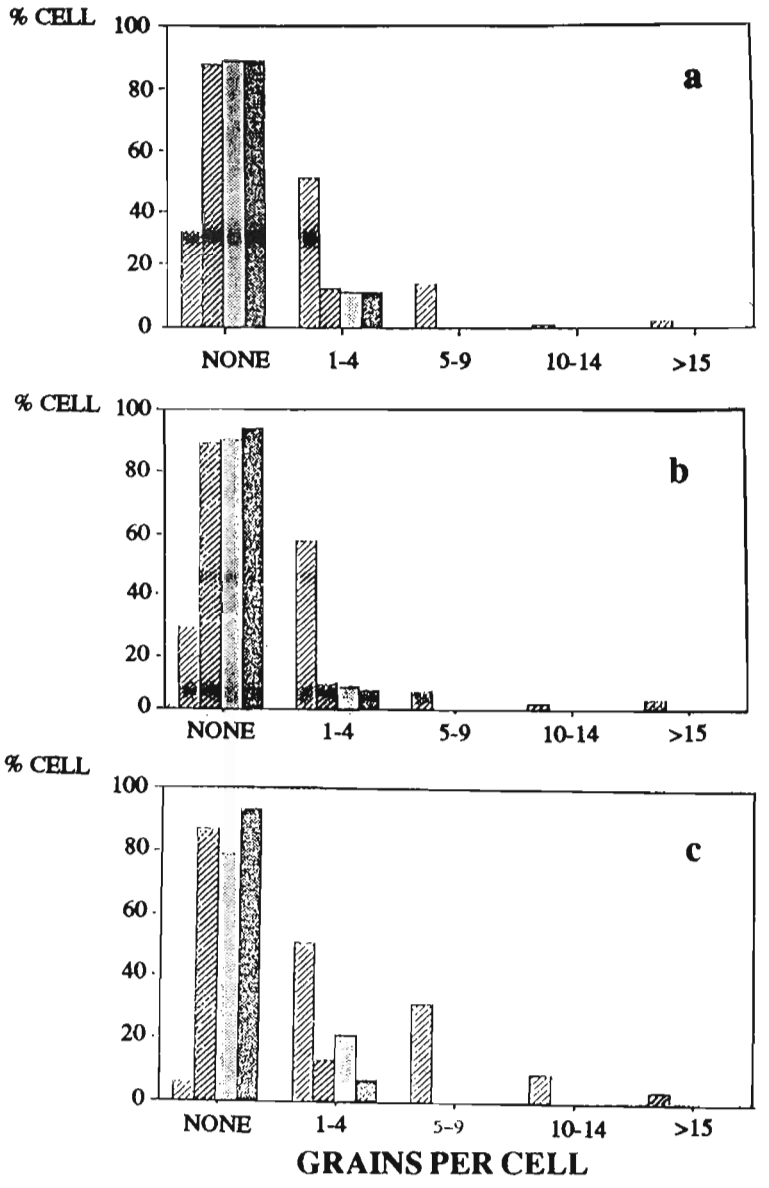


**Figure 1. Distribution of PC-1 positive cells in normal mice.**

a, spleen cells; b, lymph node cells; c, peritoneal cells
IR-518 is anti-PC-1 monoclonal antibody; WIC 79.3 is
anti *Leishmania* monoclonal antibody.

- : BALB/c cells + IR-518
- : C57B1/6 cells + IR-518
- : BALB/c cells + WIC 79.3
- : C57B1/6 cells + WIC 79.3

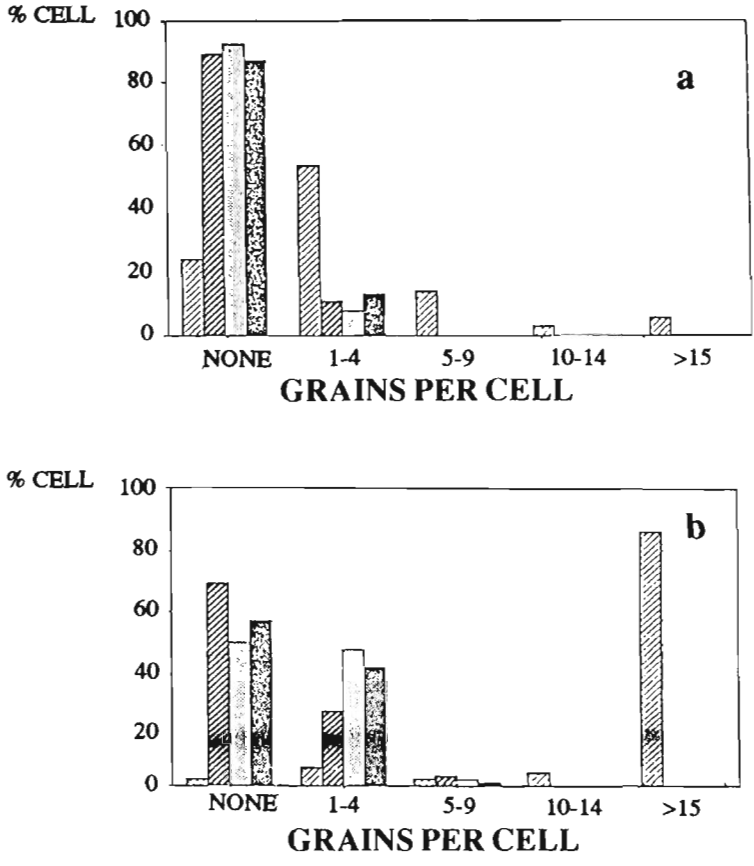


**Figure 2. Distribution of PC-1 positive cells in *M.corti*-infected mice.**

a, spleen cells; b, peritoneal cells.
IR-518 is anti-PC-1 monoclonal antibody; WIC 79.3 is anti-*Leishmania* monoclonal antibody.

: BALB/c cells + IR-518
: C57B1/6 cells + IR-518
: BALB/c cells + WIC 79.3
: C57B1/6 cells + WIC 79.3

rophages, then we cannot use PC-1 antigen as a marker for normal plasma cells.

### Nature of Cells Bearing PC-1

Overall, the following conclusion may be made. Most small lymphocytes are PC-1 negative or weakly positive. A larger proportion of large lymphocytes are positive. Cells that had the morphology of typical plasma cells were mostly, but not always positive. The large atypical cells found in the peritoneum of *M.corti*-infected mice were very strongly positive for PC-1.

The type of peritoneal cells of *M.corti*-infected mice remained unclear, because they had both plasma cell marker and macrophage markers. The possibility that they were macrophages was considered, but the absence of peroxidase activity and the staining with $(Fab')_2$ fragments of anti immunoglobulin argues strongly against this.

## REFERENCES


1. Takahashi, T., L.J., Old., and e.a. Boyse (1970) Surface alloantigens of plasma cells. J. Exp. Med. **131**: 1325-1341.
2. van Driel, I.R., A.F, Wilks., G.A. Pietersz., and J.W. Goding (1985) Murine plasma cell membrane antigen PC-1: molecular cloning of cDNA and analysis of expression. Proc. Natl. Acad. Sci. USA **82**: 1-5.
3. van Driel, I.R., and J.W. Goding., (1987) Plasma cell membrane glycoprotein PC-1. Primary structure deduced from cDNA clones. J. Biol. Chem. **262**: 4882-4887.
4. Hammerling, U., A.F. Chin., and J. Abbot (1976). Ontogeny of murine B-cell differentiation from surface-immunoglobulin-negative precursors to plasma cell. Proc. Natl. Acad. Sci. USA **73**: 2008-2012.
5. Kohler, G. and C. Milstein (1975) Continous cultures of fused cells secreting antibody of predefined specificity. Nature **256**: 495-497.
6. Stearne, P.A., I.R. van Driel., B., Grego., R.J. Simpson. and J.W. Goding. (1985) The murine plasma cell antigen PC-1: purification and partial amino acid sequence. J. Immunol. **134**: 443-448.
7. Handman, E., C.L. Greenblatt. and J.W. Goding (1984) An amphipatic sulphated glycoconjugate of Leishmania: characterization with monoclonal antibodies. EMBO J. **3**: 2301-2306.
8. Nossal, G.J.V. and O. Makela (1961) Autoradiographic studies in the immune response. I. The kinetics of plasma cell proliferation. J. Exp. Med. **115**: 209-231.
9. Nicola N. Personal communication
10. Mitchell, G.F., J.J. Marchalonis., P.M. Smith., W.L. Nicholas and N.L. Warner. (1977) Studies on immune response to larval cestodes in mice. Immunoglobulin associated with the larvae of *Mesocestoides corti*. Aust. J. Exp. Biol. Med. Sci. **55**: 187-211.

## QUESTIONS AND ANSWERS :

1. Question: Do plasma cells express PC-1 equally regardless of the isotype of immunoglobulin secreted?

   Answer : Yes, the plasma cells express PC-1 equally. There is no relation between the level of PC-1 expression and the isotype of Ig secreted.

2. Question: Is there any relationship between this PC-1 Ag with the Ig found in sera ?
   Does this PC-1 Ag have any preference to plasma cell producing certain Ig class ?

   Answer : There is no relationship between the expression of PC-1 Ag and the isotype of Ig produced by the plasma cells.

3. Question: - If we fuse plasma cell with PC-1 negative myeloma cell, will the PC-1 be expressed also by the hybridoma ?
   - Is there any mechanical relationship between the disappearance of m Fg and the expression of the PC-1 ?

   Answer : - There are no PC-1 negative myeloma cells.
   Hybridoma cells which use NS-1 cells to fuse with the spleen cells are PC-1 strong positive.
   - I do not think so. There is one cell line which has Ig but also expressed PC-1 Ag.

4. Question: For negative control why do you use another strain of mice and not the BALB mice ?

   Answer : I used different strain of mice for negative control because this strain will not produce any antigenic determinant which is recognized by anti PC-1 monoclonal antibody, since it has different PC-1 allele with BALB/c mice.
   In fact, I use also BALB/c mice as another negative control, but in this case, I react the cells from BA.