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TURNOVER AND FATE OF I-A^k ANTIGEN ON THE MURINE MACROPHAGE CELL SURFACE

Debra D. Poutsiaka, Ganesa Yogeeswaran, Douglas D. Taylor and Paul H. Black

Department of Microbiology
Boston University School of Medicine
80 E. Concord Street, Boston, MA 02118

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The macrophage plasma membrane is a major site of the cell's activities, including phagocytosis, antibody-dependent cellular cytotoxicity, and antigen presentation. To present antigen, the expression by the macrophage of immune region-associated (Ia) antigen is required. The turnover and fate of this cell surface constituent was studied in macrophages cultured with lymphokine or recombinant interferon-gamma. Surface-labeled subregion I-A^k antigen was lost from the cell surface at a rapid rate, with a half-life of approximately 24 hours. However, the shedding of I-A antigen into the culture fluid was not detected. Therefore, the loss of I-A antigen from the macrophage surface is most likely by its degradation. Upon removal of lymphokine or interferon from macrophage cultures, I-A antigen expression declined, with an apparent half-life of 2 days.

Immune region-associated (Ia) antigen is a surface glycoprotein which must be expressed at the macrophage surface for antigen presentation and, therefore, the generation of an immune response, to occur (1). The synthesis and expression of Ia antigen are inducible by exposure of macrophages to lymphokine (LK) or interferon-gamma (IFN) (2,3,4,5). The fate of surface-derived macrophage Ia antigen is uncertain. Spleen cells and B cell lymphomas shed Ia antigens in membrane-associated forms (6,7). In culture fluids of macrophages exposed to foreign antigen, a helper factor bearing determinants of Ia antigen and foreign antigen was found (8,9,10). Puri and Lonai suggested that the factor was shed from macrophages (9). However, the fate of Ia antigen of macrophages has not been studied utilizing external radiolabeling techniques.

In the present work, the fate of the subregion antigen of the macrophage Ia locus, I-A antigen, was studied using external radiolabeling techniques. I-A antigen was observed to be rapidly lost from the cell surface and did not appear to be shed.

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MATERIALS AND METHODS

Cell Culture and Assay of I-A^k Antigen Expression. The methodology for the harvest and culture of peritoneal thioglycollate-elicited macrophages and the induction, assay and immunoprecipitation of I-A^k antigen have been described in detail (11).

Radioiodination of Macrophage Surface Proteins and Glycoproteins. Macrophage membranes were radiolabeled by a modification of the lactoperoxidase-catalyzed radioiodination procedure of Cone and Marchialonis (12).

Tritiation of Macrophage Surface Glycoconjugates. Macrophage surfaces were tritiated by enzymatic oxidation followed by reduction with NaB^3H_4 (13).

Collection of Cell Lysates and Harvest Fluids. Radiolabeled macrophage cultures were terminated immediately after labeling or 8 to 48 hours later, after culturing in complete medium containing LK or IFN. Cells were lysed after rinsing dishes with PBS by the addition of 1% Triton X-100 in PBS containing 1 mM phenylmethylsulfonyl fluoride. After 30 minutes at 4°C, dishes were scraped and the lysates were pipetted repeatedly and centrifuged at 10,000g. Culture supernatants were centrifuged at 1000g to remove debris prior to analysis of released molecules. All samples were stored at -20°C until further processing.

Immunoprecipitation. The immunoprecipitation of radiolabeled cell lysates and spent culture medium utilized 10% (v/v) Protein A-Sepharose 4B (Pharmacia) in 0.05% Triton X-100, 50 mM Tris-HCl, 20 mM ethylenediaminetetraacetic acid (EDTA), 0.15 M NaCl, pH 8.0 (wash buffer) in a modification of the method used by Emerson and Cone (6). For the immunoprecipitation of I-A^k, the anti-I-A^k monoclonal antibody, 10-2.16, was used (14).

Statistical Analysis. Statistical analysis was done by student's t test. Differences were considered significant for $P<0.05$.

RESULTS AND DISCUSSION

Loss of I-A^k Antigen from the Cell Surface. Radiolabeled surface proteins or glycoconjugates of LK- or IFN-treated macrophages were cultured for up to 48 hours in the presence of LK or IFN (inducer) and subsequently cell lysates and harvest fluids were collected. In parallel cultures, the percentage of cells expressing I-A^k was determined. Lysates were immunoprecipitated for I-A^k and the results are shown in Figure 1. It is evident that after only 24 hours at least 50% of I-A^k was lost from the cell indicating that I-A^k antigen has an estimated half life (t 1/2) on the surface of approximately 24 hours. At no time was I-A^k recovered from the harvest fluid. The lowest limit of detection in the experiments, derived by calculating the lowest number of cpm's of I-A which could be detected by immunoprecipitation and comparing that value to the cpm's of material precipitated as I-A which were lost after 24 hours, was approximately 16% of the I-A antigen which was on the cell surface at 0 hours. Therefore, within our limits of detection, I-A^k antigen was not shed. In addition, shed I-A^k could not be detected from IFN-treated macrophages meta-

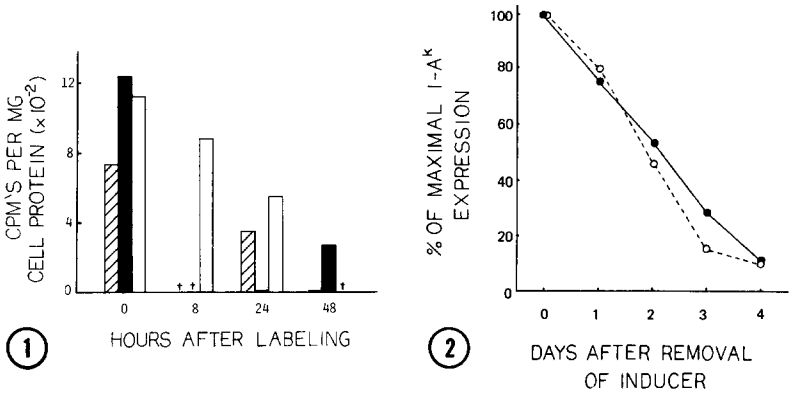

Figure 1. Loss of I-A^k Antigen from the Plasma Membrane of Macrophages Cultured with LK or IFN.

Data are expressed as cpm's of I-A^k immunoprecipitated per mg of cell lysate protein (x10^{-2}).

Experiment A (cross-hatched bars) utilized CBA/J macrophages cultured for 5 days with LK prior to radiolabeling with ^{125}I. Experiment B (solid bars) utilized CBA/J macrophages cultured with IFN for 4 days before radiolabeling with ^{125}I. Experiment C (open bars) utilized C3H/HeJ macrophages cultured with IFN for 4 days prior to labeling with ^{3}H. The percentage of cells expressing I-A^k (mean ± standard deviation), determined in parallel cultures, were: experiment A, 58.5% ± 3.3%; experiment B, 67.3% ± 6.0%; experiment C, 74.7% ± 3.8%. Cultures treated with control IFN or medium had less than 6% I-A^+ cells.
†Not determined.

Figure 2. Kinetics of the Decline of I-A^k Expression after Removal of Inducer.

The maximal values for I-A^k expression obtained from cultures treated in parallel with IFN (●——●) or LK (o---o) for the duration of the experiment (8 days) were 73.0 ± 2.0 and 50.0 ± 2.6, respectively. Cultures treated with control IFN or medium contained no more than 3% I-A^+ cells. Similar results were obtained in four additional experiments (two for each inducer).

bolically labelled with ^{3}H-glucosamine (data not shown). I-A^k was most likely degraded to an antigenically non-recognizable form. It is probable that the degradation occurred intracellularly since harvest fluid from IFN-treated macrophages was not able to degrade exogenously added radiolabeled cell lysate I-A^k after incubation for 3 hours at 37°C (data not shown).

The Loss of Expression of I-A^k Antigen after Removal of Inducer. In order to determine the fate of I-A^k antigen expression after the removal of inducer, C3H/HeJ macrophages were cultured with inducer for 4 days. Inducer was washed from parallel cultures on each of the four following days and the percentage of cells expressing I-A^k antigen was determined (Figure 2). The presence of inducer is necessary for maintenance of the I-A^+ phenotype since

even one day after its removal there was a decrease in the percentage of cells expressing I-A^k, although it was not statistically significant. The decline continued for up to 4 days after the removal of inducer at which time the percentage of cells expressing I-A^k was approximately 10% of cultures which were exposed continuously to inducer. The apparent t 1/2 of the loss of I-A^k expression in these experiments was 2 days. Since the t 1/2 of the loss of radiolabeled I-A^k molecules from the macrophage surface is approximately 24 hours, it appears that I-A^k is inserted into the plasma membrane for several days after the removal of inducer.

The inducible macrophage surface glycoprotein, I-A^k antigen, is lost from the cell surface of LK- or IFN-treated macrophages and it appears to be degraded since no shed I-A was detected in harvest fluids. It is possible that a small amount of I-A^k was shed from macrophages but was not detected in the present work given our limits of sensitivity.

Several reports have detailed the presence of a helper factor composed of a complex of Ia determinants and foreign antigenic determinants in the culture fluids of macrophages exposed to foreign antigen (8,9,10). It was postulated that the complex was shed from macrophages (9). However, in no instance were the Ia determinants of the complex directly demonstrated to be derived from the macrophage cell surface such as by study with external radiolabeling techniques. In addition, Erb et al. (8) and Friedman et al. (10) used murine peritoneal macrophages elicited by an inflammatory stimulus in the preparation of the factor. Inflammatory macrophages are known to express low levels of Ia antigen (less than 5% Ia^+ cells) (15). Therefore, Erb's and Friedman's experiments suggest that Ia antigen was shed from a predominantly Ia^- cell population. In the generation of an Ia^+ helper factor, Puri and Lonai (9) used cultures of nylon wool nonadherent spleen cells exposed to foreign antigen which were supplemented with conditioned culture medium of resident peritoneal macrophages, also known to be predominantly Ia^- (15). Although adherent spleen cells remaining in the nylon wool-nonadherent population were necessary for the production of the factor, macrophages were not identified as the source of the

Ia determinants. Therefore, it is our conclusion that none of these groups directly demonstrated the shedding of Ia antigens from macrophages. The present work clearly demonstrates the lack of shedding of Ia antigen under our conditions and suggests that its loss from the cell surface is through intracellular degradation.

The continuous presence of inducer is necessary for the maintenance of I-A^k expression since its removal causes a decline in I-A expression with an apparent t 1/2 of 2 days. Because of experiments discussed above which determined the loss of I-A molecules to occur with a t 1/2 of approximately 1 day it would be predicted that 2 days after the removal of inducer, little I-A would be present on the cell surface. The discrepancy in the results of the expression and radiolabeling experiments could be reconciled by considering several possibilities. For example, it could be due to residual I-A^k synthesis and insertion into the plasma membrane or due to a release of stored I-A^k antigen from a pool within the cell after removal of LK or IFN. In contrast, cells may be detected as I-A^+ by the microcytotoxicity assay despite a reduction in the number of I-A molecules on the surface if the minimum number necessary to achieve complement-mediated lysis is maintained.

This work has shown that the fate of induced macrophage cell surface I-A antigen is most likely intracellular degradation. The presence of Ia on the macrophage cell surface is essential for the cell's antigen presenting function. Thus, any alteration in the fate of Ia antigen may produce a change in the amount of cell surface antigen and, therefore, a change in the macrophage's immune function. For this reason, those conditions and circumstances which may alter or influence the fate of Ia antigen on the macrophage cell surface deserve further study.

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