Differential Expression of Ia Molecules by Human Monocytes

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Abstract. Human immune response genes can be divided into three distinct loci, each of which codes for three distinct families of Ia molecules: HLA-SB, HLA-DC, and HLA-DR. The tissue distribution and function of only one of these Ia molecules, HLA-DR, has been thoroughly studied. Using monoclonal antibodies, we examined the display of HLA-DR and HLA-DC molecules by adherent, human peripheral blood monocytes. The results of these studies demonstrate that although all human peripheral blood monocytes display easily detectable HLA-DR molecules, only 50% display easily detectable HLA-DC molecules. Separation of peripheral blood monocytes into HLA-DC$^+$ and HLA-DC$^-$ cells demonstrates that each population displays an equivalent density of HLA-DR molecules. Therefore, on the basis of differences in their display of these two Ia molecules, adherent peripheral blood monocytes can be divided into two broad populations: HLA-DR$^+$, HLA-DC$^+$, and HLA-DR$^+$, HLA-DC$^-$. Despite the discoordinate display of these Ia antigens, the expression of both HLA-DR and HLA-DC can be regulated by a common signal, gamma interferon (IFN-γ). Incubation of monocytes for 96 h in autologous serum leads to a marked decrease in the expression of both HLA-DR and HLA-DC. Addition of recombinant IFN-γ to the cultures leads to reexpression of both HLA-DR and HLA-DC to levels comparable to those seen in fresh monocytes. In addition, although IFN-γ does not modulate all monocyte surface markers, it can be demonstrated to modulate expression of one marker, MAC 120, in a manner similar to that observed for Ia antigens. These studies demonstrate that among human peripheral blood monocytes, the distribution of the Ia molecule, HLA-DC, is not coordinate with that of HLA-DR, although both respond to the same regulatory signal.

Introduction

Expression of Ia molecules by accessory cells, such as peripheral blood adherent monocytes (Mϕ),[1] is necessary for their ability to initiate and regulate immune reactivity (1–4). In mice, two Ia molecules, I-E and I-A, encoded for by genes within the I region have been described. In humans, three groups of molecules whose general structure resembles murine Ia molecules have been delineated, HLA-DC (DS), HLA-DR, and HLA-SB (5–13). Published studies indicate that two of these, HLA-SB and HLA-DR, have a tissue distribution similar to that described for murine Ia molecules. Both HLA-SB and HLA-DR are displayed by B cells and Mϕ (4, 11–13). In contrast to these studies, little is known about the tissue distribution of HLA-DC. We have previously provided indirect evidence indicating that although HLA-DC is present on the surface of Mϕ, only ~50% of peripheral blood Mϕ are HLA-DC$^+$ (14). This suggests that there is heterogeneity among peripheral Mϕ in regards to Ia expression. Furthermore, other studies from our laboratory have demonstrated that the human monocytoid cell line U-937 can be induced to express HLA-DR. This expression represents a pretranslational event induced by human recombinant gamma interferon (IFN-γ, 15). In those experiments, expression of HLA-DC was never seen with the expression of HLA-DR. This suggests that not only may there be discoordinate expression of HLA-DR and HLA-DC in normal Mϕ but that the regulatory signals governing expression of these Ia antigens may be different. Studies in the murine system have demonstrated that both I-A and I-E can be induced by a soluble factor released by activated T cells (16–21). One of these factors has been demonstrated to be

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1. *Abbreviations used in this paper:* CM, culture media; IFN-γ, human recombinant gamma interferon; Mϕ, peripheral blood adherent monocyte; PBMC, peripheral blood mononuclear cells; MCF, mean channel fluorescence.

IFN-γ (22–23). In the human, however, only modulation of HLA-DR expression by supernatants of activated T cells and IFN-γ has been studied (24–27).

In the present study, we investigated the heterogeneity of Mϕ Ia expression directly by using a monoclonal antibody specific for HLA-DR, two monoclonal antibodies specific for HLA-DC molecules, and a rabbit heterosera specific for HLA-DC molecules. We demonstrate that there is discoordinate expression of HLA-DR and HLA-DC among normal human peripheral monocytes. However, despite discoordinate expression, both Ia molecules appear to be under the regulatory control of the lymphokine IFN-γ. We also demonstrate that IFN-γ can modulate the expression of another Mϕ marker, MAC 120.

Methods

Antibodies. CA 206 is an IgG2b mouse monoclonal antibody that is specific for nonpolymorphic determinants of HLA-DR molecules (14). Rb03 is a rabbit serum that is directed against Ia molecules whose amino acid sequence is distinct from HLA-DR and homologous to murine I-A (5, 6, 14). These molecules have been termed HLA-DS or DC (6, 7) and for convenience will be called HLA-DC in this paper. Leu 10 is an IgG1 mouse monoclonal antibody, which was a gift from the Becton-Dickinson Monoclonal Center, Inc., Mountain View, CA. Leu 10 was derived from the fusion of mouse NS-1 cells with spleen cells of BALB/c mice immunized with tonsillar B cells (Chen, Y. X., F. Brodsky, R. L. Evans, and N. L. Warner; manuscript in preparation). It reacts with B cells of all DR specificities except for homozygous DR 3 and DR 7 cells and the molecule it depicts is distinct from HLA-DR. On the basis of competitive binding and sequential immunoprecipitation, Leu 10 appears to react with HLA-DC molecules (28). Studies from our laboratory indicate that Leu 10 immunoprecipitates an Ia molecule distinct from HLA-DR and electrophoretically identical to that precipitated by a known anti HLA-DC sera, Rb03 (Gonwa, T., and J. Stobo; unpublished observations). Genox 3.53 is a mouse monoclonal antibody known to be directed against an HLA-DC molecule associated with HLA-DR 1, 2, and 6 (8). This molecule has an amino acid sequence distinct from HLA-DR, identical to that precipitated by Rb03, and homologous to murine I-A (7). It was the gift of Dr. F. Brodsky (Becton-Dickinson & Co., Mountain View, CA). The mouse IgM monoclonal antibody MAC 120 identifies a 120,000-D molecule displayed on the surface of a subset of Mϕ that are important in antigen presentation (29). The expression of this Mϕ marker has been previously demonstrated to correlate with HLA-DC (DS) expression (14). This antibody is also known as Leu M2 (Becton-Dickinson & Co.) and was used as supplied by the company. Leu M3 is a mouse IgG monoclonal antibody that reacts with mature monocytes (30); it was the gift of Becton-Dickinson & Co. For controls, the IgG myeloma protein MOPC 195 or the IgM myeloma protein MOPC 104E (Bionetics Laboratory Products, Kensington, MD) was used.

Fluorescent analysis was performed by using an indirect immunofluorescent technique. Cells to be analyzed were incubated with the first antibody under saturating conditions for 30 min at 4°C, washed, and incubated with saturating amounts of either an $F(ab')_2$ fluoresceinated goat anti-mouse antibody or an $F(ab')_2$ fluoresceinated goat anti-rabbit Ig antibody. Controls consisted of cells incubated with either an isotype matched myeloma protein or normal rabbit serum in the first step followed by the fluoresceinated second antibody (Cappel Laboratories, West Chester, PA) for 30 min at 4°C. Cells were then washed twice over 5% bovine serum albumin, resuspended in phosphate-buffered saline, and analyzed by flow cytometry using log amplification on an FACS IV (Becton-Dickinson & Co.). This technique has been previously described (14).

Isolation and culture of peripheral blood monocytes. Peripheral blood mononuclear cells (PBMC) were obtained from normal donors and adhered on plastic petri dishes as previously described (29). Nonadherent cells were removed by vigorous washing. Adherent cells were removed with lidocaine as described (29) and these constituted the adherent peripheral blood monocyte (Mϕ) population (>95% esterase positive). In the culture experiments described, Mϕ were suspended in RPMI-1640 media with 10% heat-inactivated autologous serum and cultured on bacteriological petri dishes (Falcon Labware, Oxnard, CA) at 37°C in a humidified 95% O_2:5% CO_2 atmosphere. For some experiments, Mϕ were incubated in the presence of conditioned media (CM) derived from activated T cells as a source of Ia inducing material. This was prepared as previously described (15). Briefly, PBMC were incubated at a concentration of 0.5×10^6 cells/ml in RPMI 1640 supplemented with 10% fetal calf serum and concanavalin A (15 μg/ml, ICN Nutritional Biochemicals, Cleveland, OH) for 48 h at 37°C. The cells were removed by centrifugation, the concanavalin A was removed by absorption of the CM with sepharose 4B (Pharmacia, Uppsala, Sweden), and the CM was stored at −70°C until use. For some experiments, Mϕ were incubated in the presence of human recombinant gamma interferon isolated from *Escherichia coli* (IFN-γ, the gift of Genentech Inc., South San Francisco, CA).

Results

Adherent Mϕ are one cell type capable of presenting antigen to T cells (14, 29, 31, 32). This requires the display of appropriate Ia molecules. We and others have previously demonstrated that all Mϕ display easily detectable HLA-DR molecules (14, 31, 33, 34). Previous studies from this laboratory provided indirect evidence suggesting that only ~50% of peripheral blood Mϕ also display easily detectable HLA-DC molecules (14). To directly analyze the display of HLA-DC by Mϕ, saturating amounts of Leu 10 were used to stain peripheral blood Mϕ from normal donors. A typical experiment is depicted in Fig. 1. All Mϕ display easily detectable HLA-DR molecules. However, only ~50% display reactivity with Leu 10, which is greater than the reactivity noted with the control myeloma protein (MOPC 195). Therefore, only 50% of Mϕ are HLA-DC positive when compared with control. Similar results were obtained by using two other known anti-DC reagents, Rb03 and Genox 3.53, and the peripheral blood Mϕ from individuals of several different HLA-DR phenotypes. (Genox only stains Mϕ from individuals who are HLA-DR 1, 2, or 6.)

In no instance was the frequency of cells expressing HLA-DC molecules noted to be greater than the frequency of cells expressing HLA-DR molecules. One explanation for this apparent discrepancy of Ia antigen expression could be the state of maturation of the Mϕ. Incubation of murine Mϕ in supernatants from activated T cells (CM) or IFN-γ increases

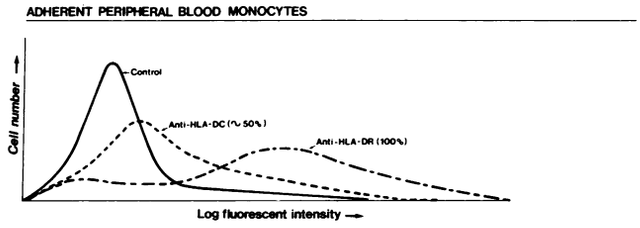

Figure 1. Analysis of HLA-DR and HLA-DC expression by adherent peripheral blood Mϕ. Mϕ were obtained as detailed in Methods and analyzed for reactivity with the control myeloma protein MOPC 195 (control), CA 206 (anti HLA-DR), or with Leu 10 (anti HLA-DC). The cells were initially reacted with saturating amounts of the first antibody, washed, and incubated with saturating amounts of a fluoresceinated $F(ab')_2$ goat anti-mouse Ig. The percentage of cells reacting with the antibodies greater than control is indicated in the parentheses. The results are representative of six experiments in separate individuals, performed with three different anti-HLA-DC reagents: Leu 10, Genox 3.53, and Rb03.

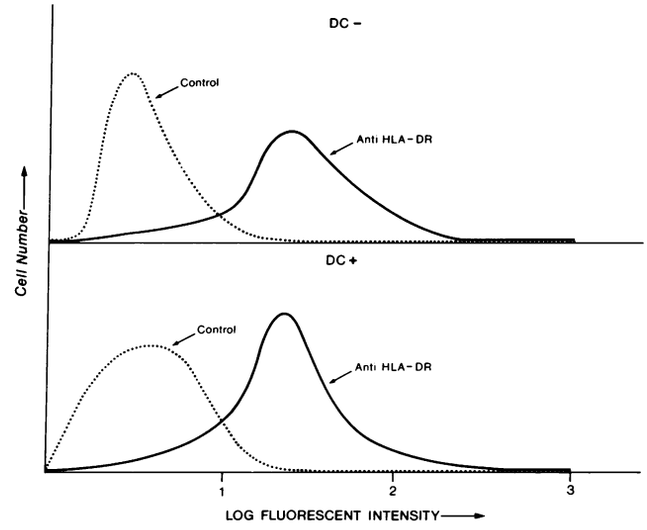

Figure 2. Mϕ expression of HLA-DR does not correlate with the expression of HLA-DC. Mϕ were separated into HLA-DC^+ and HLA-DC^- populations by sorting on an FACS. Both populations were incubated overnight in 10% autologous serum and then analyzed by flow cytometry for reactivity with the control myeloma protein MOPC 195 (control) or CA 206 (anti HLA-DR) as described in Fig. 3. This is representative of two experiments in HLA-DR disparate individuals.

expression of Ia. Therefore, we incubated freshly isolated Mϕ in human CM for 24 h and then assayed the expression of HLA-DR and HLA-DC. After this incubation, although HLA-DR expression increased slightly, the number of HLA-DC^+ Mϕ remained the same (data not shown). The CM used in these experiments was active in that it could induce the expression of Ia molecules by a human monocytoid cell line. Therefore, although all Mϕ appear to be HLA-DR^+, only 50% display easily detectable HLA-DC molecules and this cannot be increased by soluble materials capable of enhancing Ia expression.

It could be argued that Mϕ defined as HLA-DC^- actually display too few HLA-DC molecules to be detected by flow cytometry. This question was addressed in two sets of experiments. First, assuming coordinate expression of these Ia molecules, one might expect these HLA-DC^- cells would also express a low density of HLA-DR. To examine this possibility, Mϕ were stained with Leu 10 and sterilely sorted into HLA-DC^+ and HLA-DC^- populations by using an FACS IV. HLA-DC^+ included all cells staining greater than the MOPC control. HLA-DC^- consisted of the remaining population. The HLA-DC^+ and HLA-DC^- populations were then incubated overnight in autologous serum and reanalyzed for the expression of both HLA-DC and HLA-DR. Restaining the populations for expression of HLA-DC after an overnight incubation demonstrated that the HLA-DC^- population remained HLA-DC^- (no cells staining greater than control), while the HLA-DC^+ population remained HLA-DC^+ (all cells stained greater than control, data not shown). The two populations were analyzed for the expression of HLA-DR determinants by using the monoclonal anti-HLA-DR antibody, CA 206. As can be seen in Fig. 2, the frequency of HLA-DR positive cells as well as the density of HLA-DR per cell were identical in each population. Separating the HLA-DC^+ and HLA-DC^- populations utilizing the anti-DC1 monoclonal antibody Genox 3.53 gave identical results. Thus, HLA-DR expression by HLA-DC^+ and HLA-DC^- Mϕ is equivalent. The expression of these Ia molecules is not coordinate.

Secondly, the number of HLA-DR and HLA-DC molecules present on the cell surface of Mϕ was quantitated in two individuals by using flow cytometry with log amplification (35–37). Fluorescent staining with MOPC 195 (control) gave a mean channel fluorescence (MCF) of 30 and 31. Fluorescent staining with CA 206 (anti HLA-DR) gave an MCF of 83 and 84, respectively. In these experiments, the calibration curve generated indicated that a log difference in fluorescence was 62 channels. Thus, the technique can measure up to a log difference in fluorescent intensity and because the experiments were performed under saturating conditions, this implies a log difference in molecules. In the same experiment, the MCF of the population of Mϕ that would be considered HLA-DC^-, i.e., staining no greater than control, was determined by using utilizing Leu 10 as the anti-HLA-DC reagent. These values were 38 and 39, respectively. Using the above mentioned calibration curve, this indicates that the average density of HLA-DC molecules in the HLA-DC^- population was 20–25% of the density of HLA-DR molecules. The MCF of the HLA-DC^+ population determined with Leu 10 was 78 and 78, no different than staining with CA-206. The average number of HLA-DR molecules present on Mϕ is 120,000/cell (37). Thus, the HLA-DC^- Mϕ may express on the average no more than 24,000–30,000 HLA-DC molecules. We have previously dem-

onstrated that these cells do not function as effectively in antigen presentation as the DR^+ DC^+ Mϕ (14).

To determine if the absence of HLA-DC expression among a subpopulation of Mϕ is a stable phenotype, we studied changes in expression of this molecule which might occur during culture. This has been demonstrated to mimic Mϕ maturation (38, 39). Mϕ were incubated in 10% autologous serum and at 24 and 96 h analyzed for HLA-DR and HLA-DC expression. As demonstrated in Fig. 3, at no time during culture was there an increase in HLA-DC expression and by 96 h, HLA-DC expression had almost entirely disappeared. At this time, the frequency of HLA-DR^+ Mϕ had also decreased.

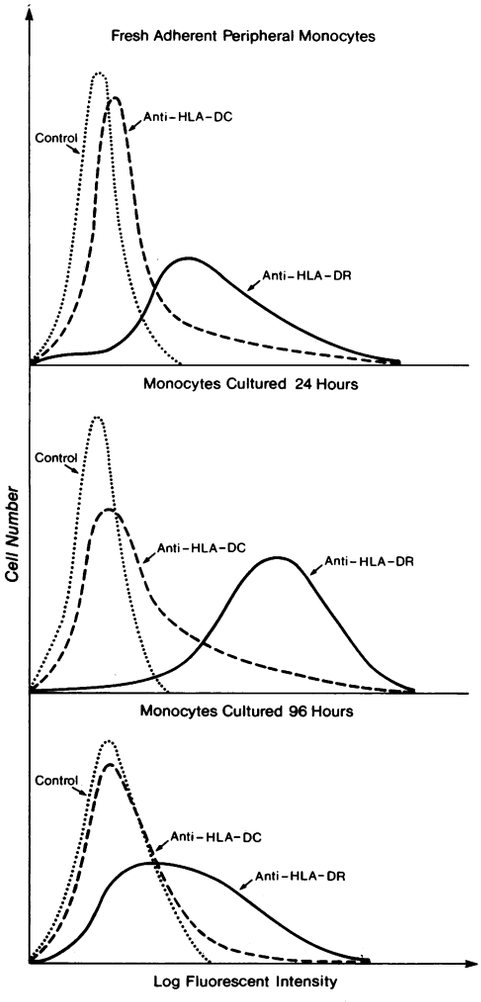

Figure 3. Expression of HLA-DR and HLA-DC by cultured Mϕ. Peripheral blood Mϕ were cultured in autologous serum for the times indicated. They were then harvested and analyzed for their reactivity with Leu 10 (anti-HLA-DC), CA 206 (anti-HLA-DR), or the myeloma protein MOPC 195 (control) by using indirect immunofluorescence and flow cytometry as described in Fig. 3. This is representative of two experiments.

Therefore, the failure of 50% of the Mϕ to display HLA-DC molecules does not relate to a time dependent maturation event which can be mimicked by culture.

One explanation for the discoordinate expression of HLA-DR and HLA-DC by Mϕ could be that they are under separate regulatory control. To investigate this, we used cultured Mϕ as a source of Mϕ whose Ia expression could be increased. Mϕ were obtained from normal donors and placed in culture with autologous serum. At 7 d, fresh autologous serum with or without human recombinant IFN-γ (100 IU/ml) was added to the cultures. 48 h later, cells were harvested, washed, and analyzed for expression of HLA-DR and HLA-DC. At the same time, freshly prepared Mϕ from the same donor were assayed for expression of HLA-DR and HLA-DC. Results of a typical experiment are shown in Fig. 4. Cultured Mϕ lose expression of most of their HLA-DR and all of their HLA-DC. After incubation in IFN-γ, the expression of both Ia antigens is increased to the density present on fresh Mϕ. Similar results were obtained by using CM instead of IFN-γ. This suggests that the factor present in CM that induces HLA-DR and HLA-DC is IFN-γ. This finding was demonstrated by using Genox 3.53, Leu 10, and Rb03. Further experiments indicated that HLA-DR and HLA-DC could be induced with as little as 10 IU/ml of IFN-γ. However, 50–100 IU/ml was optimum with amounts >100 IU/ml inducing no greater increase in Ia antigen expression. Therefore, for most experiments 100 IU/ml of IFN-γ was used. Experiments performed with the monocyte cell lines U-937 and HL-60 have indicated that increase in Ia expression induced by IFN-γ can be detected as soon as 4 h after addition of IFN-γ (15, and footnote 2). Maximal expression was at 48 h. Thus, although Mϕ are discoordinate in their expression of HLA-DR and HLA-DC, both Ia antigens appear to be under the regulatory control of the same lymphokine, IFN-γ.

We next investigated changes in two other Mϕ markers induced by culture. Leu M3 is a mouse monoclonal antibody that reacts with mature monocytes (30). Leu M2 is the mouse monoclonal antibody MAC 120. This antibody reacts with a subset of peripheral monocytes that are important in antigen presentation. Expression of MAC 120 has been correlated with expression of HLA-DC (14). Mϕ were isolated from a normal donor and placed in culture with autologous serum. After 48 h, fresh medium with or without IFN-γ (10 IU/ml) was added to the cultures. This concentration of IFN-γ was used based on preliminary experiments that indicated that 10 IU/ml of IFN-γ was optimal for inducing monocyte markers in the monocyte line HL-60.[2] 48 h later, the cells were harvested and assayed for expression of Leu M3 and MAC 120. Freshly isolated Mϕ from the same donor were isolated and analyzed for Leu M3 and MAC 120. A typical experiment (representative of three done in different individuals) illustrates the results of

2. Gonwa, T. A., B. M. Peterlin, and J. D. Stobo. Unpublished observations.

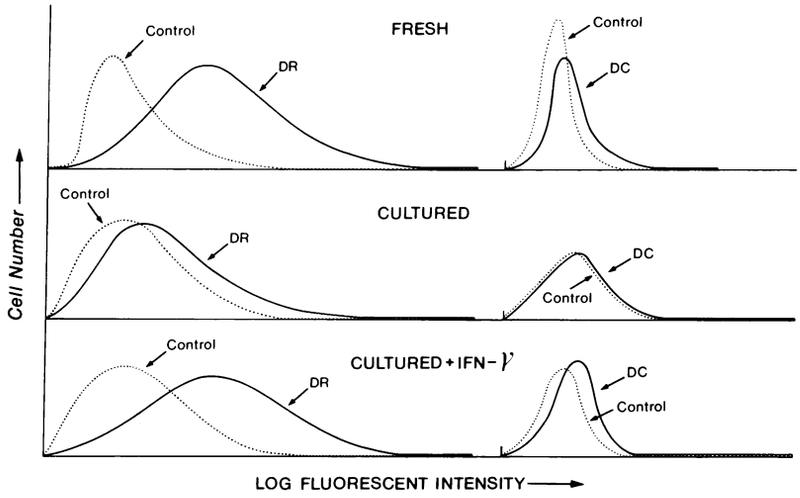

Figure 4. IFN-γ modulates HLA-DR and HLA-DC expression in normal human monocytes. Freshly isolated Mϕ (fresh), Mϕ cultured for 7 d (cultured), and 7-d cultured Mϕ incubated for an additional 48 h with 100 IU/ml of IFN-γ (cultured ± IFN-γ) from the same donor were analyzed for reactivity with the myeloma protein MOPC 195 (control), the monoclonal antibody CA 206 (anti HLA-DR), or the monoclonal antibody (Genox 3.53 (anti HLA-DC) by using indirect immunofluorescence under saturating conditions and flow cytometry with log amplification. The results presented are representative of three experiments performed with two different anti-HLA-DC reagents: Leu 10 and Genox 3.53.

this experiment (Fig. 5). Expression of Leu M3 does not change with culture, nor is its expression modulated by IFN-γ. Although it appears that the expression of Leu M3 has increased in the cultured Mϕ, note that the staining with control antibody has also increased. This increase in autofluorescence is characteristic of cultured cells. The difference between staining with control and with Leu M3 in the two populations is not different. Thus, not all Mϕ surface markers decrease during culture nor do they all appear to be under the regulatory influence of IFN-γ. In contrast, MAC 120 displays a pattern similar to that seen with Ia antigens. Culture of Mϕ results in disappearance of MAC 120 expression. MAC 120 expression is lost as soon as 24 h after initiating culture. Exposure of the cultured Mϕ to IFN-γ results in the reexpression of MAC 120. Thus, the expression of this Mϕ marker, which has previously been correlated with expression of HLA-DC, also appears to be under regulatory control. The cell surface antigen with which MAC 120 reacts is shared by platelets (N. Warner, personal communication). Therefore, one concern is that the expression of MAC 120 by Mϕ merely represents platelet satellitism. The above experiments demonstrate that MAC 120 is indeed expressed by Mϕ as they were done with serum that had been centrifuged at 35,000 *g* for 10 min before use. This procedure removes all platelets and platelet membrane microparticles (40). The reexpression of MAC 120 induced by purified IFN-γ represents synthesis by Mϕ and cannot be ascribed to absorption of platelets or platelet membrane microparticles onto the Mϕ.

Discussion

The recent availability of molecular probes for genes in the human major histocompatability complex has advanced our knowledge concerning the organization and structure of immune response genes and their products. It is clear that there exist three distinct human immune response loci that code for three families of Ia molecules, HLA-DC, HLA-DR, and HLA-SB. Furthermore, each of these loci may contain multiple copies of the genes coding for α- and β-chains (5–14, 41–44). Studies of the expression, tissue distribution, fine structure, and function of each family of human Ia molecules has been hampered by the paucity of reagents reacting with individual molecules. In this study, we use monoclonal antibodies to examine the tissue distribution of two Ia molecules. One of these antibodies, CA 206, reacts with molecules coded for by genes within the HLA-D locus: HLA-DR molecules (14, 32). The other antibodies, Leu 10, Genox 3.53, and Rb03 depict a molecule (HLA-DC) that has an amino acid sequence distinct from HLA-DR molecules and is encoded for by distinct genes (5–9, 28). The studies demonstrate that although all Mϕ display classical HLA-DR, only approximately half display a substantial density of HLA-DC. These findings confirm our previous studies suggesting heterogeneity among peripheral Mϕ in regards to their display of Ia molecules (14). In a previous report, we demonstrated that peripheral blood Mϕ could be divided into two populations based on their display of a molecule depicted by the antibody MAC 120. MAC 120^+ Mϕ displayed both HLA-DR and HLA-DC molecules. MAC 120^- Mϕ were in contrast $HLA\text{-}DR^+$ and $HLA\text{-}DC^-$. We have previously demonstrated functional differences between these two populations of Mϕ (14).

It is not clear from these studies whether or not there are some Mϕ that are truly $HLA\text{-}DC^-$. It is clear that some Mϕ stain with anti-HLA-DC reagents no brighter than the control myeloma protein. This suggests that they are either HLA-DC negative or display very low density of HLA-DC molecules. As indicated in the results, the $HLA\text{-}DC^-$ Mϕ could display 24,000–30,000 HLA-DC molecules on average compared with 100–120,000 HLA-DC molecules present on $HLA\text{-}DC^+$ Mϕ. However, the data presented in Fig. 2, showing that $HLA\text{-}DC^+$ and $HLA\text{-}DC^-$ Mϕ have comparable densities of HLA-DR molecules, support the concept that there need not be

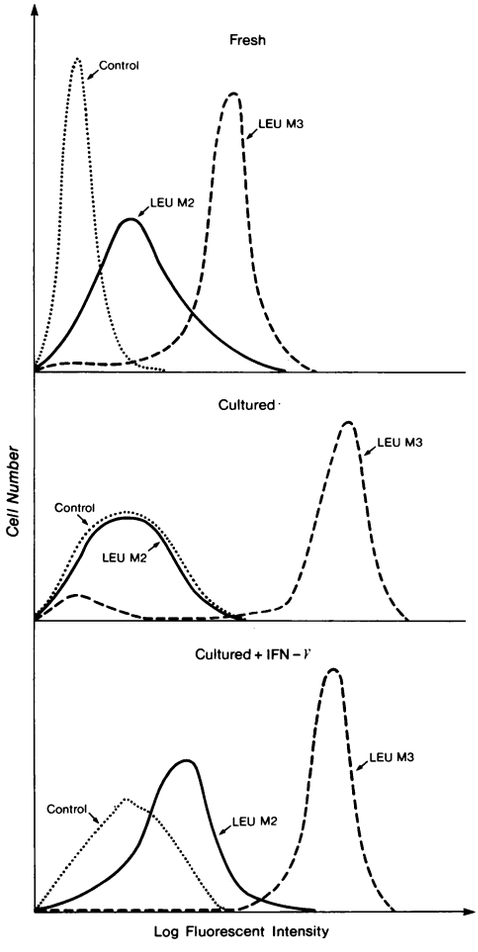

Figure 5. IFN-γ modulates expression of Leu M2 (Mac-120) in normal monocytes. Freshly isolated Mϕ (fresh), Mϕ cultured for 4 d (cultured), or 4-d cultured Mϕ incubated an additional 48 h with 10 IU/ml of IFN-γ were analyzed for reactivity with a myeloma control protein (control), the monoclonal antibody Leu M2, or the monoclonal antibody Leu M3 by using indirect immunofluorescence and flow cytometry with log amplification. The control for Leu M2 was MOPC 104E and the control for Leu M3 was MOPC 195. These gave equal staining of the cell populations and are represented by one line.

coordinate display of Ia molecules by Mϕ. Similar discoordinance of expression has been demonstrated for murine Ia molecules. Three studies demonstrate that murine Mϕ differ in their expression of I-A and I-E (45–47). Huber and Rosenwasser (48) have demonstrated that the sex-linked Ia determinant Ia.W39 was expressed on some but not all splenic Mϕ (48). Furthermore, recent studies using human pre-B cell lines indicate that HLA-DR is expressed before the expression of HLA-DC (49). This suggests that the initial signals for expression of these Ia molecules may be different. Whether or not the differential expression of Ia molecules and the existence of Mϕ subsets are stable phenotypes is not clear. The studies outlined in Fig. 3 demonstrate that DC^- Mϕ do not become DC^+ after 4 d in culture. Moreover, it is possible to demonstrate that incubation of fresh normal Mϕ in IFN-γ, a process potentially capable of increasing both HLA-DR and HLA-DC expression, does not result in an increase in the percentage of $HLA\text{-}DC^+$ Mϕ (data not shown). It should be noted that at 24 h, the density of HLA-DR on Mϕ increased slightly when compared with fresh Mϕ. This has been reported by others (33, 50). However, continuation of cultures beyond 24 h leads to a decrease in HLA-DR expression. This initial increase may represent residual soluble factors either secreted by contaminating lymphocytes or present in small amounts in human serum. The gradual decrease of Ia antigen expression may represent the depletion of these factors necessary for Ia antigen expression. These studies demonstrate that the expression of Ia antigens by Mϕ is not a static event but requires constant regulatory signals that modulate expression.

Despite discoordinate expression of HLA-DR and HLA-DC among human Mϕ, the data presented in Fig. 4 demonstrate that the display of both molecules can be regulated by the same signal, IFN-γ. The level of this control has been demonstrated to be pretranslational for expression of HLA-DR in the human monocyte cell line U-937 (15), the myelomonocytic cell line HL-60,[2] and in human B lymphoid and melanoma cell lines (51). Molecular mechanisms of IFN-γ control of HLA-DC expression are under study. Thus, similar to the murine system, IFN-γ appears to regulate all classes of Ia expression (22, 23).

Not all surface markers are under regulatory control by IFN-γ. The data presented in Fig. 5 demonstrate that culture or IFN-γ has no effect on the expression of the mature monocyte marker Leu M3. In contrast, the expression of MAC 120 (defined by Leu M2) can be enhanced by IFN-γ. This finding also demonstrates conclusively for the first time that Mϕ express MAC 120 directly rather than stain with this antibody because of platelets adhered to their cell surface (platelet satellitism). Confirmation of this finding can be found in experiments that demonstrate that purified IFN-γ induce MAC 120 in previously negative HL-60 cells[2] and that the vitamin D metabolite 1,25-dihydroxy vitamin D_3 induces expression of MAC 120 in HL-60 cells (52, 53). Why this surface marker should be under regulatory control similar to Ia antigens is unknown. It is interesting that MAC 120 correlates with HLA-DC expression (14) and that it marks a functionally distinct subset of Mϕ. Further studies of the relationship of this Mϕ membrane protein to HLA-DC are in progress.

The demonstration of discoordinate expression but similar control by IFN-γ of Ia molecules by human Mϕ is important for several reasons. For example, it indicates that accessory cells that express HLA-DR may not express other Ia molecules important in determining their accessory cell function. This consideration is important for studies comparing the relative

accessory cell functions of isolated cell populations. Moreover, it provides a model for investigating the molecular events regulating HLA-DR and HLA-DC gene expression. Although IFN-γ can induce expression of both molecules, other molecular events are obviously important in determining gene expression. The nature of these molecular events is presently under investigation.

Acknowledgments

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