

# Splenic Macrophages From the NOD Mouse Are Defective in the Ability to Present Antigen

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IDDM results from the destruction of pancreatic  $\beta$ -cells by autoreactive T-cells that appear to avoid deletion early in development, possibly due to improper interaction with antigen-presenting cells (APCs) resident in the thymus or periphery. In the nonobese diabetic (NOD) mouse, there exists a defect in APC function characterized by its failure to fully mature upon stimulation. The NOD mouse thus provides an excellent model for the investigation of APC dysfunction and development and how these relate to the incidence of autoimmune diabetes. We initiated studies of APC function in the NOD mouse with respect to antigen processing and presentation, using a well-characterized antigen hen egg lysozyme (HEL) and comparing it with the closely related, major histocompatibility complex (MHC) (I-A<sup>g7</sup>) identical, diabetes-resistant mouse strain NOR. Proliferation assays comparing NOD and NOR HEL-specific T-cells demonstrated that the T-cell proliferation response of the NOD mouse to both native and denatured forms of the antigen is lower than that of NOR. When crisscross proliferation experiments were conducted using purified T-cells and irradiated spleen cells as APCs from both strains, the results demonstrated that the defect in proliferation resided in the APC compartment of activation. The levels of intracellular glutathione (GSH) were compared in splenic macrophages from NOD and NOR mice; it was found that on antigenic stimulation, NOR macrophages produced significantly more intracellular GSH than did NOD macrophages, even under hyperglycemic (50 mmol/l glucose) conditions. The lower amount of GSH seen in the NOD may result in less efficient processing of antigen, and subsequently, lower levels of T-cell activation. *Diabetes* 47:1212-1218, 1998

**S**usceptibility to IDDM, an organ-specific autoimmune disease, is strongly linked to specific major histocompatibility complex (MHC) haplotypes in humans (1). The nonobese diabetic (NOD) mouse is a widely used animal model for human IDDM. As with human MHC haplotypes, the unusual H-2<sup>g7</sup> MHC (K<sup>d</sup>, I-A<sup>g7</sup>, I-

E<sup>null</sup>, D<sup>b</sup>) of this mouse is strongly associated with diabetes susceptibility (2-4). However, it has been determined that diabetogenesis in both humans and NOD mice results from complex polygenic interactions between several alleles within the MHC and multiple susceptibility genes outside of the MHC (2-6).

Several lines of evidence from the NOD mouse model suggest that non-MHC-linked diabetes susceptibility genes contribute to subtle abnormalities in monocyte differentiation. These susceptibility genes and the diabetogenic MHC haplotype lead to the generation of antigen-presenting cells (APCs) that are unable to process and/or present  $\beta$ -cell autoantigens in a tolerogenic fashion but retain the capacity to activate autoreactive T-cells in the periphery. For example, it has been demonstrated that marrow-derived APC (but not thymic epithelial cells) from NOD congenic mice expressing the diabetes-resistant H-2<sup>nb1</sup> haplotype of NON mice could inhibit the development of diabetogenic T-cells from NOD marrow (7). It has also been shown that the inability of NOD APC to activate immunoregulatory T-cells in a syngeneic mixed-lymphocyte reaction was associated with homozygous expression of H-2<sup>g7</sup> (8). The defect in antigen presentation may be due to the inability of the NOD APC to respond to growth factors and thus produce fully mature macrophages. This is indicated by studies showing that in response to the myeloid growth factor colony-stimulating factor 1 (CSF-1), there is a reduced level of promonocyte proliferation and development to mature macrophages in NOD mice, unlike in diabetes-resistant NON and SWR mice (7). Further evidence for defective APCs was provided by the fact that bone marrow-derived macrophages fail to respond to  $\gamma$ -interferon (INF- $\gamma$ ) stimulation in a normal fashion (9). In diabetes-resistant strains, INF- $\gamma$ , which induces macrophage differentiation and activation, inhibits stimulation of bone marrow cells by CSF-1. In the NOD mouse, however, this inhibitory effect of INF- $\gamma$  is abrogated (9).

It has also been reported that APCs stimulated with INF- $\gamma$ , granulocyte macrophage-CSF, or the mitogen lipopolysaccharide (LPS) demonstrate an increase in intracellular thiols, such as glutathione (GSH) (10,11). GSH is an ubiquitous tripeptide thiol involved in a variety of cellular functions (10). It serves as a coenzyme, protects cells from oxidative damage, and affects initiation and progression of lymphocyte activation (12). With respect to immune system function, GSH may be a limiting factor in that it can potentiate certain T-cell functions *in vivo* (10). In addition, during the processing of antigen in the lysosomes to produce immunogenic peptides, an essential step is the reduction of disulfide bonds within the antigen; GSH is also involved in the reduction of disulfide

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APC, antigen-presenting cell; CFA, complete Freund's adjuvant; CSF-1, colony-stimulating factor 1; GSH, glutathione; HEL, hen egg lysozyme; INF- $\gamma$ ,  $\gamma$ -interferon; LPS, lipopolysaccharide; MHC, major histocompat-

properly to cytokine signals may have an effect on its capacity to process antigen in an efficient manner.

In summary, antigen presentation in the NOD mouse is dysfunctional with respect to proper maturation and development of macrophages. The inability of these APCs to respond appropriately to growth factors/cytokines leaves the NOD with a defect in its immune repertoire that may result in the inefficient processing and presentation of antigens and, subsequently, less-than-optimal activation of T-cells. It has been shown that antigens that are processed and presented inadequately can stimulate T-cells in the periphery but are unable to induce tolerance (14,15). Furthermore, it is thought that a more highly activated APC is required for the activation of immunoregulatory Th2 T-cells than is necessary for activation of Th1 T-cells (16–18). The inability to properly process and present  $\beta$ -cell antigens in a manner sufficient to cause the deletion or tolerization of autoreactive T-cells, together with the deficiency in activation of immunoregulatory cells, may leave the NOD mouse with only the capability to generate effector T-cell responses leading to an autoimmune state.

To date, studies on defective antigen presentation in the NOD mouse have primarily involved genetic manipulation and investigation of cytokine function. We investigated the APC defect by looking at processing and presentation events with a defined antigen, hen egg lysozyme (HEL), which has been well studied in other mouse strains and is considered a model protein for antigen processing (19–21). As the APC, we used splenic macrophages, which are considered to be “professional APC” and are well described in terms of their antigen-presenting function. Using the HEL system, we analyzed macrophage antigen-presenting function in the NOD mouse and compared it with macrophages from the closely related diabetes-resistant NOR mouse. In contrast to the NOD mouse, the NOR/Lt mouse is an insulinitis-resistant and diabetes-free strain produced from an isolated genetic contamination within a NOD pedigree line by the C57BL/KsJ (BKS) strain (22,23). Although the NOR is diabetes resistant, it shares the diabetogenic H-2<sup>g</sup> and, as is observed in the NOD, peritoneal macrophages are unable to secrete normal levels of interleukin-1 on LPS stimulation (23).

## RESEARCH DESIGN AND METHODS

**Mice.** Eight-week-old male NOD/Bdc mice were obtained from the colony at the Barbara Davis Center for Childhood Diabetes. Eight-week-old male NOR/Lt mice were purchased from Jackson Laboratories (Bar Harbor, ME).

**Measurement of T-cell proliferation to HEL.** NOD and NOR mice were immunized at the base of the tail with 120  $\mu$ g of native HEL emulsified in complete Freund's adjuvant (CFA). At 7 days after immunization, the animals were killed, and the inguinal and periaortic lymph nodes were removed for isolation of T-cells. The nodes were homogenized and then resuspended in 10 ml of Clicks culture medium containing 1% Nutridoma SP serum supplement (Amersham), 2% L-glutamine, 1% gentamycin, and 0.02% 2-ME (Clicks/Nutridoma). For bulk population T-cell proliferation assays, lymph node T-cells were plated in decreasing concentrations ( $8-2 \times 10^5$  cells/well) into 96-well round-bottom, antigen-coated plates, containing either no antigen (media control) or 250  $\mu$ g/ml native or denatured HEL, in a total volume of 200  $\mu$ l Clicks/Nutridoma. This concentration of antigen was determined to be optimal, and no signs of toxicity were observed in responder cell populations. After incubation at 37°C for 4 days, the cells were pulsed with 0.5  $\mu$ Ci of <sup>3</sup>H-TdR for 18 h, and then harvested onto glass fiber filters with an automated sample harvester. Radioactive measurements were made on a  $\beta$ -scintillation counter.

Denaturation of HEL antigen was accomplished by dissolving 0.5 g HEL into 35 ml of a solution containing 8 M urea and 0.2 M 2-ME and stirring at room temperature overnight. The reduced, denatured antigen was then alkylated with 0.3 M

dialyzed against several changes of 5 mmol/l phosphate-buffered saline, pH 7.4, followed by 0.1 mmol/l (NH<sub>4</sub>)<sub>2</sub>HCO<sub>3</sub>; then it was lyophilized.

**Crisscross in vitro proliferation assay.** NOD and NOR mice were immunized with 120  $\mu$ g of native HEL in CFA, as described above. At 7 days after immunization, the animals were killed, and the inguinal and periaortic lymph nodes were removed for isolation of T-cells. The lymph node T-cells were purified from individual mice using CD4<sup>+</sup> Celllect columns (Edmonton, Alberta, Canada) following the manufacturer's specifications. The purified T-cells were then titrated into wells of 96-well round-bottom, antigen-coated plates containing either no antigen or 250  $\mu$ g/ml native or denatured HEL, in a total volume of 200  $\mu$ l Clicks/Nutridoma medium. Either NOD or NOR autologous irradiated splenic cells were added to the T-cells in a crisscross fashion to serve as APCs. Assays were pulsed with 0.5  $\mu$ Ci of <sup>3</sup>H-TdR for 18 h and harvested as described above.

**Preparation of splenic macrophages.** Spleen cell suspensions from NOR or NOD mice were pooled within groups, and NH<sub>4</sub>Cl was added to lyse the erythrocytes. The spleen cells were incubated in sterile Petri plates in culture medium (Dulbecco's modified Eagle's medium with supplements) containing 10% fetal calf serum for 1.0 h at 37°C, after which the nonadherent cells were gently washed away. The adherent cells were removed with the use of nonenzymatic dissociation buffer (Life Technologies, Grand Island, NY). The remaining cells were then washed and resuspended in Clicks/Nutridoma.

**Determination of intracellular GSH.** Based on methods described by Tietze (24), determination of intracellular GSH was made in splenic macrophages of both NOD and NOR mice after the addition of LPS, HEL, or medium alone. Splenic macrophages were prepared as described above and were plated at a concentration of  $1 \times 10^6$  cells/well with LPS (10  $\mu$ g/ml), HEL (250  $\mu$ g/ml), or medium alone. They were then incubated at 37°C for 24 h. The cells were washed 2 $\times$  with phosphate-buffered saline and resuspended in 2.5% sulfosalicylic acid ( $1 \times 10^6$  cells/0.4 ml) to precipitate cellular protein (11). The precipitated protein was isolated from the supernatant by centrifugation (12,000 rpm for 5 min) and assayed for protein content. For intracellular GSH determination (GSH assay), the cell supernatant fraction was neutralized with triethanolamine (6  $\mu$ l/0.1 ml sample volume) and then mixed with 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent), GSH reductase, and NADPH. The resulting color product was measured as a change in absorbency at 405 nm by a multiscan plate reader. Samples were run against standards containing known amounts of GSH to generate a concentration curve.

**Effects of increased glucose on intracellular GSH levels.** Splenic macrophages were plated at a concentration of  $1 \times 10^6$  cells/well with either LPS (10  $\mu$ g/ml) or HEL (250  $\mu$ g/ml). Control wells consisted of purified macrophages alone. Glucose (50 mmol/l) and s-methyl GSH (2 mmol/l) were added in combination or separately to all wells. The cells were treated as previously described for determination of GSH and protein content.

**Statistical analysis.** Statistical analyses were done by two-tailed Student's *t* test using the InStat software package to determine differences within the means of the sample groups.

## RESULTS

**Comparison of NOD and NOR T-cell proliferation responses to native and denatured HEL after immunization with native HEL.** To determine whether splenic APCs from NOD mice are capable of processing antigen with the same efficiency as those from the diabetes-resistant NOR strain, antigen-specific T-cell proliferation experiments were performed. After immunization of NOD and NOR mice with native HEL, lymph node T-cell responses to denatured or native antigen were assessed. (Total cell numbers of lymph node preparations from NOD and NOR mice were very comparable. The proportion of T-cells were also determined to be comparable, as determined by equivalent T-cell yields after purification for crisscross experiments). Results in Fig. 1 indicate that with decreasing numbers of responding T-cells, the proliferation response to both native and denatured antigen is markedly lower and declines more precipitously with NOD T-cells than with NOR T-cells. In this particular experiment, the NOR response was two- or threefold higher at three different concentrations of T-cells than that observed with NOD T-cells. Although the magnitude of responses between assays was variable, the same general trend was seen in repeated tests. The greater proliferation with NOR T-cells

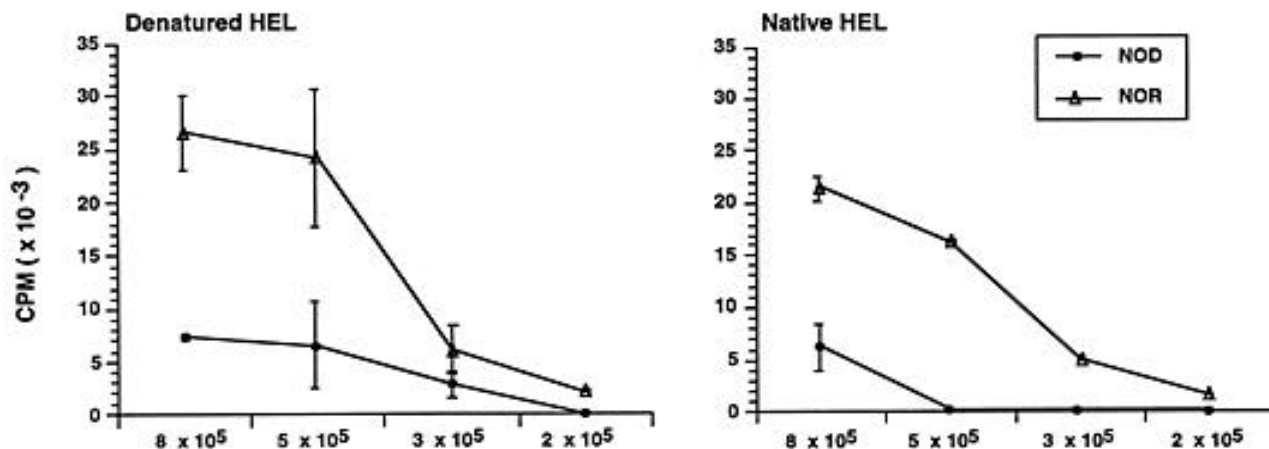


FIG. 1. Comparison of T-cell responses from NOD and NOR mice to native and denatured HEL. NOD and NOR mice were injected with 120  $\mu$ g native HEL in CFA. Periaortic and inguinal lymph node cells were harvested from the animals 7 days after immunization and were plated at the represented concentrations of lymph node cells with the addition of autologous irradiated spleens cells to equalize cell density. The lymph node cells were pulsed with either native or denatured HEL at 250  $\mu$ g/ml. On day 4 of culture, the lymph node cells were pulsed with ( $^3$ H-TdR) for 18 h and harvested. Background counts per minute for non-antigen-pulsed wells was <10% of that of the antigen-pulsed wells. The data is representative of repeated experiments.

(where, in some assays, NOD T-cell responses are not even detectable), suggesting that NOR splenic APCs are more efficient at processing and presenting antigen, which likely results in an increased number of NOR antigen-specific T-cells. The results of Fig. 1 also demonstrate that even if the antigen is partially denatured (through reduction of disulfide bonds and treatment with 8 M urea), NOD splenic APCs do not stimulate T-cells in vitro at the same level as NOR splenic APCs.

**Comparison of NOD and NOR T-cell proliferation responses to HEL with NOD and NOR APC.** To determine whether the lower NOD T-cell proliferation responses illustrated in Fig. 1 were due to an inability of NOD splenic APC (or a subset of these APC) to process antigen as efficiently as spleen cells from the NOR mouse, a crisscross experiment was performed in which both NOD and NOR T-cell responses were measured with both NOD and NOR spleen cells (whole spleen cell populations or enriched populations of macrophages or B-lymphocytes). For these experiments, mice were immunized with HEL. After removal of the lymph nodes, T-cells were purified by affinity chromatography on CD4+ Celllect columns or by Mouse CD4 (L3T4) Dynabeads. (The yields of purified T-cells were comparable from both NOD and NOR mice.) For assay, responder T-cells from each mouse strain were combined with whole irradiated spleen cells, enriched splenic macrophages, or enriched nonirradiated B-lymphocytes of each mouse strain. In Fig. 2, the data are shown for presentation by splenic macrophages, and it is evident that NOR macrophages present antigen more efficiently than NOD macrophages, regardless of whether presentation was to NOD or NOR HEL-specific T-cells. Presentation by whole irradiated spleen cell populations (data not shown) was very similar, with spleen cells from NOR presenting more efficiently than spleen cells from NOD. However, there was no difference in presentation between NOD and NOR enriched nonirradiated B-lymphocyte populations (data not shown). The difference in magnitude of T-cell responses between the experiments

concentration,  $5 \times 10^5$  cells per well, of NOR T-cells) may be partially due to the presence of a functional B-lymphocyte population in the whole lymph node populations used in Fig. 1. Also, the responding T-cells in Fig. 1 were unmanipulated, whereas both T-cell and APC populations in Fig. 2 were subjected to purification procedures that often affect the overall magnitude of responses in these types of assays. This experiment indicates that the defect in the NOD T-cell proliferation response results from less efficient antigen presentation by NOD splenic macrophages (but not by NOD B-lymphocytes) and is not due to an inherently lower capacity of NOD T-cells to proliferate.

**GSH levels in purified splenic macrophages from NOR and NOD mice.** The T-cell proliferation experiments established that NOD splenic macrophages were less efficient than NOR splenic macrophages in processing and presentation of antigen, resulting in lower proliferation responses with NOD antigen-specific T-cells. Because studies by others have shown that GSH is critical for correct processing of antigens (10,13) and because it has also been established that cytokines and LPS stimulation can enhance GSH production in APC (11,25), splenic macrophages were purified as described in METHODS and were assayed for intracellular GSH after stimulation with LPS or HEL antigen. The results, illustrated in Fig. 3, demonstrate that upon stimulation with antigen or LPS, NOR splenic macrophages have a greater amount of basal GSH than do macrophages from the NOD mouse. The most dramatic difference between NOR and NOD macrophage GSH levels was observed in macrophages stimulated by antigen (in this case, HEL): intracellular levels of GSH in NOR macrophages were significantly higher ( $P < 0.002$ ) than those in NOD. Lower GSH levels could explain, at least in part, why NOD splenic macrophages are inferior to NOR splenic APCs in the activation of antigen-specific T-cells.

**Effects of increased glucose and s-methyl GSH on intracellular levels of GSH.** A hyperglycemic environment

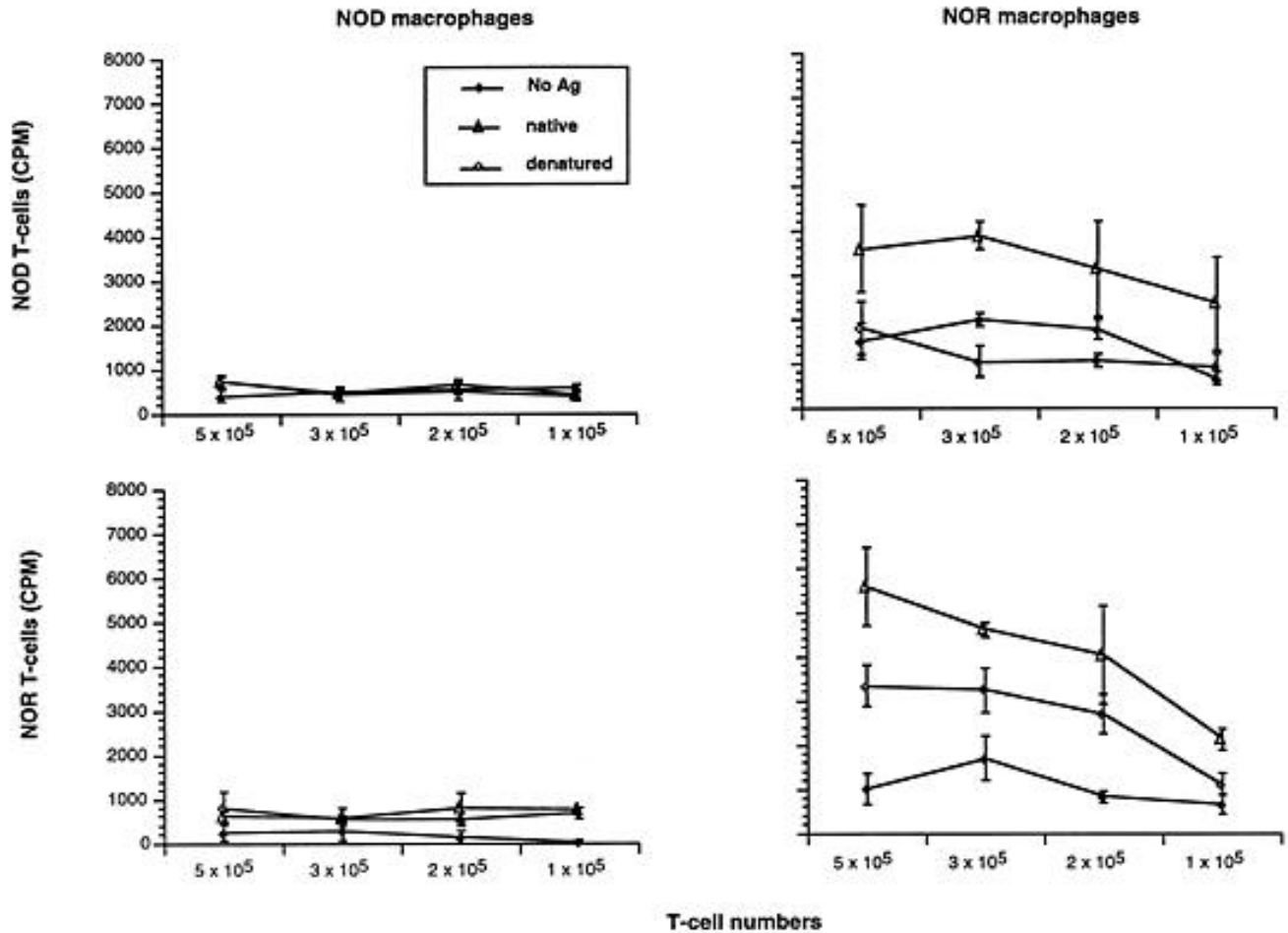


FIG. 2. T-cell responses to native and denatured HEL comparing NOD and NOR APCs. NOD and NOR mice were injected with 120  $\mu$ g native HEL in CFA. Periaortic and inguinal lymph node cells were harvested from the animals 7 days after immunization. The T-cells were purified from individual mice and were plated at the T-cell concentrations indicated, with the addition of purified splenic macrophages, from either NOD or NOR in a crisscross fashion, to serve as APCs. The cultures were pulsed with either native or denatured HEL (250  $\mu$ g/ml), and on day 4 of culture, the plates were pulsed with (3H-TdR) for 18 h before harvest. The data is representative of three different experiments.

icals, which cause cell damage and destruction (26–28). Glutathione peroxidase is a key enzyme involved in reducing these oxygen radicals to less damaging species, a process that occurs at the cost of intracellular GSH. We therefore investigated the effect of hyperglycemia on the levels of intracellular GSH in splenic macrophages unstimulated or combined with LPS or HEL (Fig. 4). Incubation of NOR and NOD purified splenic macrophages with 50 mmol/l glucose resulted in very little change from basal levels of intracellular GSH in both NOR and NOD animals, with respect to all treatments, control, LPS, and HEL. S-methyl GSH is a GSH ester precursor of GSH and will increase its intracellular levels. The addition of 2 mmol/l s-methyl GSH under the same high-glucose conditions (50 mmol/l glucose + GSH ester) resulted in the NOR macrophages showing a threefold increase over NOD macrophages in intracellular GSH in controls (Fig. 4A) and a five- and threefold increase over NOD macrophages in response to stimulation by LPS and HEL, respectively (Fig. 4B and C). The addition of 2 mmol/l s-methyl GSH in the absence of high glucose also resulted in higher levels of intracellular GSH in NOR macrophages than in NOD macrophages under all three conditions. These data indicate that when challenged

was inferior to the NOR with respect to regulating intracellular GSH levels, even in the presence of the GSH ester.

## DISCUSSION

We have reported here on studies of APC function in the NOD mouse through the investigation of T-cell responses to a defined antigen, HEL, and comparing these results with those obtained from the closely related, non-diabetes-prone NOR mouse. Our data show that T-cell responses from HEL-primed mice are considerably lower in NOD mice than in NOR mice and that this defect resides in the splenic APC, not the T-cell. When NOD T-cells were combined with NOR spleen cells as APCs, the T-cell responses to HEL, native and denatured, were comparable to the NOR T-cell responses with NOR APC. To investigate a possible mechanism for the lower antigen-presenting capacity of NOD spleen cells, macrophages were isolated from spleen cell suspensions of unimmunized NOD or NOR mice and assayed for GSH content after stimulation with a mitogen (LPS) or with the antigen HEL. The results showed that NOR macrophages had higher levels of GSH than did NOD macrophages upon stimulation with LPS; an even more dramatic difference was observed

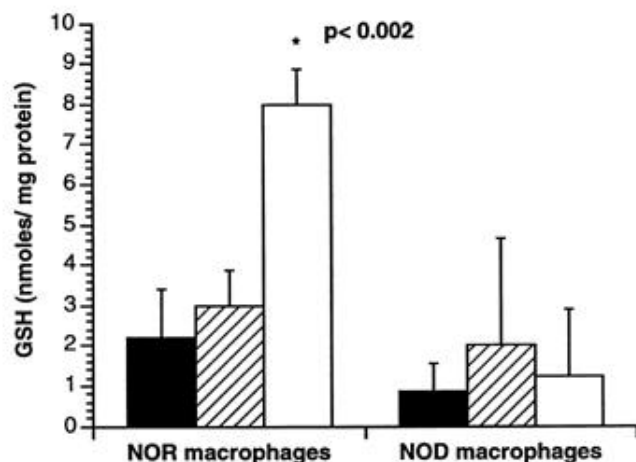


FIG. 3. Levels of GSH in splenic macrophages from NOD and NOR mice. The bars indicate the concentration of GSH in purified splenic macrophages from NOD and NOR mice treated with medium alone (■), LPS (▨), or HEL (□) for 24 h. Intracellular GSH was measured against standards containing known amounts of GSH. Data are mean values + SE of four experiments. *P* values are shown for conditions where statistical significance was noted between treatments.

i.e., 50 mmol/l glucose, the GSH levels were reduced in both cell populations, but with the addition of the GSH precursor s-methyl GSH they were restored to higher levels in NOR macrophages than in NOD macrophages.

It has been reported that the NOD APC has a defect in maturation (9), and this developmental deficiency may be accompanied by an inability of the NOD APC to generate GSH upon cytokine or antigen stimulation. In a high-glucose environment, conditions that might be expected to exist in the hyperglycemic animal or patient, GSH production is decreased even further. Also, chronic hyperglycemia in the diabetic animal or patient leads to oxidative stress, manifested by a low ratio of reduced to oxidized GSH (27). A reduced level of GSH may affect antigen processing and presentation. It is known that during the processing of antigen in the lysosome to produce immunogenic peptides, the reduction of disulfide bonds is essential and dependent on cytosolic cysteine and GSH (13). Cysteine reduces disulfide bonds within the protein and allows for the unfolding of the protein, thus providing easy access by proteolytic enzymes that cleave the protein into peptides to be bound by class II MHC molecules before presentation (29). The oxidized form of the amino acid cysteine requires GSH for reduction back to cysteine. Further evidence indicating that reduction of disulfide bonds is critical in the processing of antigens comes from studies on minimal requirements for protein binding to class II. At low pH and under conditions favoring disulfide bond reduction, it was found that the native form of the antigen HEL could inhibit binding of a labeled HEL peptide to a purified class II protein at pH 5 in the presence, but not the absence, of the reducing agent dithiothreitol (29). Thus, a decrease in intracellular GSH levels can lead to lowered capacity for reduction of disulfide bonds and negatively affect antigen processing. In another recent report, Chinese hamster ovary (CHO) cells transfected with murine class II genes were found to exhibit a unique antigen-processing defect consisting of an impaired ability to process antigens

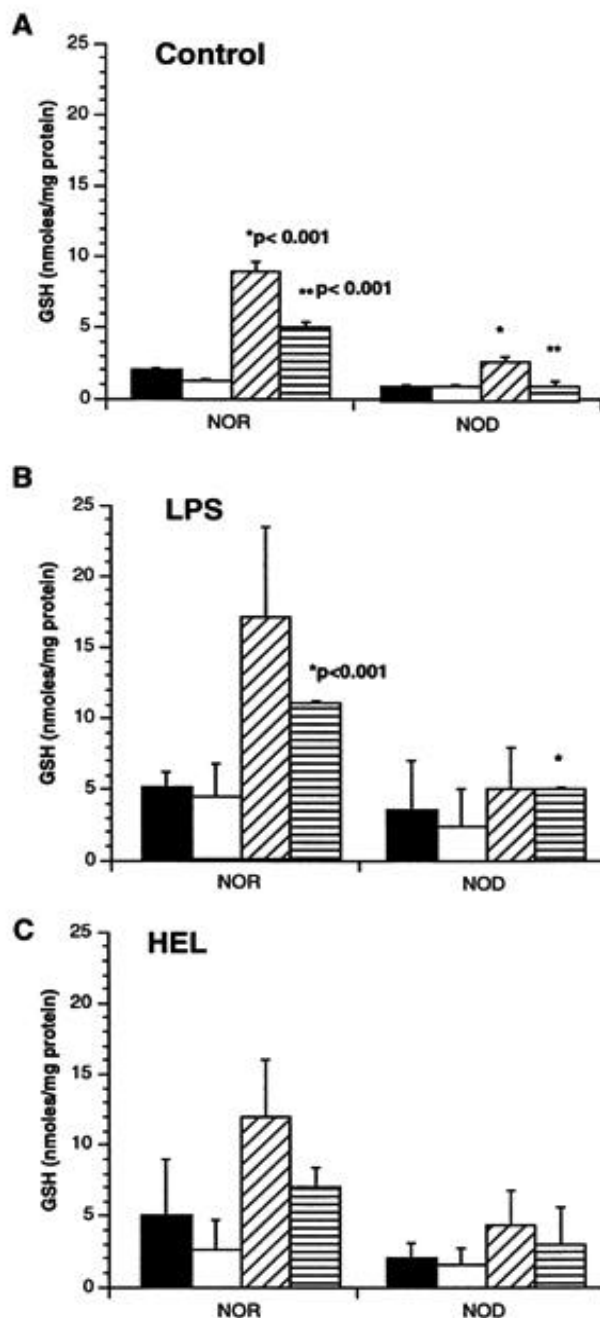


FIG. 4. Effects of increased glucose and s-methyl GSH on GSH levels in NOD and NOR splenic macrophages. The bars show the concentration of GSH in purified splenic macrophages from NOD and NOR mice treated with medium control alone (A), LPS (B), or HEL (C). In addition, each of these culture conditions were supplemented with medium only (basal levels, ■); with 50 mmol/l glucose (□); with 50 mmol/l glucose and 2 mmol/l s-methyl GSH (GSH ester; ▨); or with 2 mmol/l s-methyl GSH (▩). Intracellular GSH was measured against standards containing known amounts of GSH. Data are mean values + SE of four experiments. *P* values are shown for conditions where statistical significance was noted between treatments.

lower levels of GSH (30). The quantity of intracellular GSH therefore appears to be critical in determining the efficiency of processing protein antigens containing disulfide bonds.

In the presence of splenic macrophages from NOD mice, T-

even with partially denatured antigen. This result suggests that there may be insufficient protein processing in NOD splenic macrophage, manifested by reduced proteolytic cleavage and thus limiting the number of peptides to be presented. Further evidence that the NOD mouse is defective in its ability to process and present antigen was provided by the work of Hansen et al. (31) with GAD 65, in which it was shown that synthetic GAD peptides elicited a strong T-cell response when used as immunogens, but no T-cell responses to these same peptides could be detected if the native antigen was used in immunization. The fact that not all peptides that can be generated from an antigen are necessarily presented under normal conditions was also demonstrated in studies by Mamula (32) on whole cytochrome c versus a peptide produced by cyanogen bromide cleavage. Furthermore, it was found that immunization with the immunogenic peptide together with the native protein could break T-cell tolerance to other sites on the protein (32). Another possibility is that, in some cases, full-length proteins can bind directly to class II (33). If partially denatured peptides from intact antigens are bound to class II in a mouse that is unable to process antigen normally, the resulting antigen-MHC complexes can be more heterogeneous than those produced by intracellular processing of intact protein antigens (34). As Mamula and Craft (14) pointed out, T-cells specific for cryptic peptides may exist because APCs are unable to process and present these peptides normally; this may be the situation in the NOD mouse.

Another factor contributing to the inefficient antigen presentation by NOD spleen cells could be the inherent inability of the NOD APC to bind peptide in the class II molecule tightly enough to insure that the peptide-class II complex reaches the cell surface. The unique I-A<sup>97</sup> of NOD has been reported to be SDS unstable and a poor binder of peptides, which might thus reduce the number of peptides presented (35). This is an important point in view of the fact that a more stable MHC class II molecule is more likely to interact with T-cells and stimulate activation. However, the NOD mouse and the NOR mouse share the same MHC class II (H-2<sup>97</sup>), but the NOR splenic APCs were more efficient at processing and presenting antigen to the NOR T-cells. Therefore, poor class II binding in itself is not a sufficient explanation for the defect in antigen processing present in the NOD APC. It appears, then, that in the NOD mouse, a developmental defect in splenic macrophages, together with the diabetogenic MHC haplotype, results in an APC that is unable to process and/or present autoantigens in a tolerogenic fashion but retains the capacity to activate autoreactive T-cells in the periphery.

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