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INFLUENCE OF H-2 HAPLOTYPES ON POLY IC INDUCTION OF XANTHINE OXIDASE AND POLY IC INDUCED DECREASES IN P-450 MEDIATED ENZYME ACTIVITIES

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Polyriboinosinic - polyribocytidylic acid (poly IC), a potent interferon inducer, induced xanthine oxidase 24 hours after treatment with 5 mg/kg ip to different degrees among four H-2 congenic mice ($P < 0.05$): B10 (H-2b: 236 ± 27% of the control value) > B10.RIII (H-2r: 171 ± 29%) = B10.F (H-2n: 161 ± 12%) > B10.BR (H-2k: 136 ± 15%). Aryl hydrocarbon hydroxylase (AHH) activity showed an inverse correlation with inducibility of xanthine oxidase ($r = -0.71$, $P < 0.01$). However, there were no significant changes in activities of heme pool associated enzymes, such as catalase, tryptophan pyrrolase and d-aminolevulinic acid synthase in these mice. H-2 haplotype seems to have an influence on poly IC induction of xanthine oxidase thereby causing a decrease in AHH. © 1986 Academic Press, Inc.

It has recently been found that polyriboinoisinic-polyribocytidylic acid (poly IC), a potent interferon inducer, and interferon (INF) per se (α/β) induced xanthine oxidase (1-4). It is known that induction of xanthine oxidase after treatment with Newcastle disease virus (NDV) is influenced by the IFN locus through regulation of IFN induction (2). Each individual INF locus seems to respond to specific immunological stimuli. For example, the IF-1 locus regulates the induction of INF with Newcastle disease virus (NDV) but

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does not regulate those inductions either with cytomegalovirus (6) or with poly IC (10).

Recently we reported that antigenic stimulation of mice with sheep red blood cells induces xanthine oxidase in an H-2 haplotype related manner (8). It is known that macrophages activated by phagocytosis of antigens, or lymphocytes activated by antigenic stimulation, excrete IFNs (9). Thus our finding might imply that H-2 haplotype has some degree of influence on IFN induction. If so, the question arises whether H-2 might influence poly IC induction of IFNs and/or of xanthine oxidase.

The principal aim of the present study was to test the possibility that the H-2 haplotype influences the inducibility of xanthine oxidase following treatment with poly IC. An apparent IF-1 haplotype related decrease in P-450 mediated enzyme (MFO) activities has also been reported following NCV treatment and assumed to result from differences in inducible amounts of xanthine oxidase (2). If so, then the inducibility of xanthine oxidase might also explain the apparent influence of H-2 on decreases in MFO activities after poly IC. Thus our second aim was to test the possibility that differences in degree of induction of xanthine oxidase in H-2 congenic mice following poly IC might underlie or correlate with the degree of decrease in P-450 dependent enzymes. In this study, the enhancement of immunostimulation, as measured by a plaque forming cell assay (PFC) to sheep red blood cells (SRBC), was also investigated in order to determine if H-2 type influences immunostimulation after treatment with poly IC.

MATERIALS AND METHODS

Animals

Ten-month-old H-2 congenic male mice of strains C57Bl/10 Sn (H-2b), C57Bl/10.F (H-2n), B10.BR/SgSn (H-2k), and B10.BRIII (7INS)/Sn (H-2r), hereafter B10, B10.F, B10.BR and B10.RIII, were used. These strains are of C57Bl/10 background and are reported to be IF-$1^{h/h}$ (5). All mice were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained in our animal facility. Poly IC (Sigma Chemical Co., St. Louis, MO) dissolved in 0.9% NaCl was intraperitoneally administered to animals at a dose of 5 mg/kg (2.0 ml/kg) around 9:00 am. Controls received 0.9% saline solution. Test and control animals were sacrificed by cervical dislocation 24 hours later. The number of test and control animals was 5 for each species except B10.F (n = 4).

In Vitro PFC Assay

The animals were sacrificed 24 hours after treatment with poly IC and the spleens removed and teased apart in cold Hank's balanced salt solution (HBSS). Cell number was adjusted to 10^7 cells/ml (10^6 cells/well). The cells were sensitized with 5 x 10^6 SRBC/well and cultured for 4 days in RPMI 1640 supplemented with 20% fetal calf serum (FCS), antibiotics, 2 mM glutamate and 1 mM sodium pyruvate. On day 5, lymphocytes were prepared and hemolytic plaques were assayed in triplicate cultures as described before (8). The variations in these assays were within 10%.

Preparation and Enzyme Assays

Livers were removed immediately after sacrifice and homogenized in ice cold 20 mM Tris·HCl/0.25 M sucrose, pH 7.4 buffer in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at differnt speeds to obtain mitochondrial, postmitochondrial and microsomal fractions as described previously (10).

Xanthine oxidase exists in two interconvertible forms: an oxidase (Type O) and as an NAD-dependent dehydrogenase (Type D). The cytosol fraction (105,000 g supernatant fraction) was dialysed at 4°C for 24 hours against 1.15% KCl to remove inhibitors and assayed for Type O oxidase (XO) activity by following urate formation at 292 nm without NAD, and for Type D dehydrogenase (XD) with NAD and measuring NADH formation at 340 nm by the method of Della Corte and Stirpe (14). Total xanthine oxidase is defined as the combined activities of XO and XD. Contents of P-450 and activity of cytochrome P-450 reductase in microsomes were determined by the methods of Omura and Sato (12) and Yasukochi and Masters (13), respectively. The activities of the microsomal P-450 dependent enzymes, arylhydrocarbon hydroxylase (AHH), 7-ethoxycoumarin O-deethylase (7-EC) and p-Nitroanisole O-demethylase (P-NA) were measured as described previously (8).

δ-Aminolevulinic acid synthetase (ALA-S) and tryptophan pyrrolase were determined by the methods described previously (10). Catalase activity was measured by the method of Bergmeyer using a 600g supernatant fraction (14).

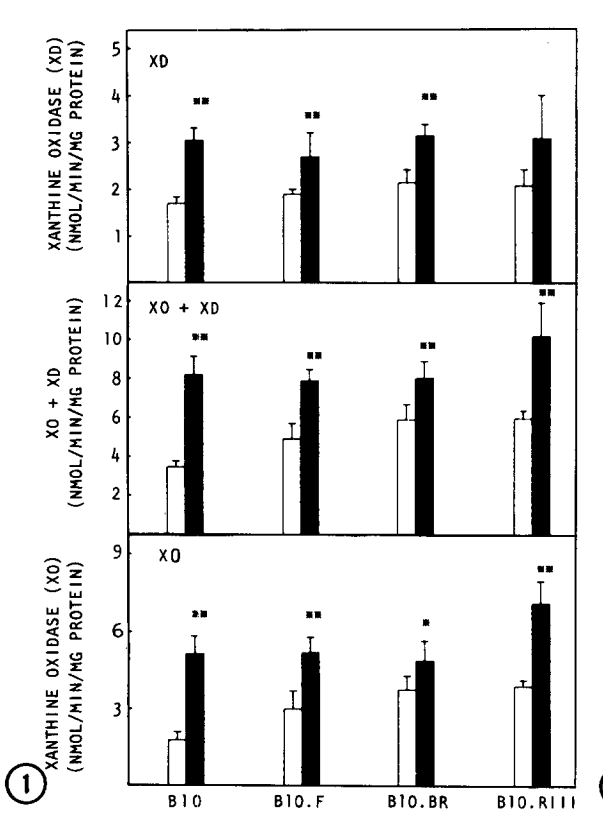
Statistics

All data were calculated as means and standard deviations. Comparisons of mean values between treated and control animals were made by using the Student t-test, and those among the four strains by using one-way analysis of variance followed by Duncan's multiple range test. A P value < 0.05 was considered as significant unless specified.

RESULTS

Influence of Poly IC on Xanthine Oxidase Activity

Poly IC increased the activities of xanthine oxidase Type O (XO) and Type D (XD) (Figure 1). The inducibility of xanthine oxidase was well segregated by H-2 haplotype. Increases in total xanthine oxidase activity (XO + XD; Mean ± SD) was 236 ± 27% of the control value in B10 (H-2b), 171 ± 29% in B10.RIII (H-2r), 161 ± 12% in B10.F (H-2n) and 136 ± 15% in B10.BR (H-2k). The degree of induction was different among congenic strains: B10 (H-2b) > B10.F (H-2n) = B10.RIII (H-2r) > B10.BR (H-2k) (Duncan's multiple range test).



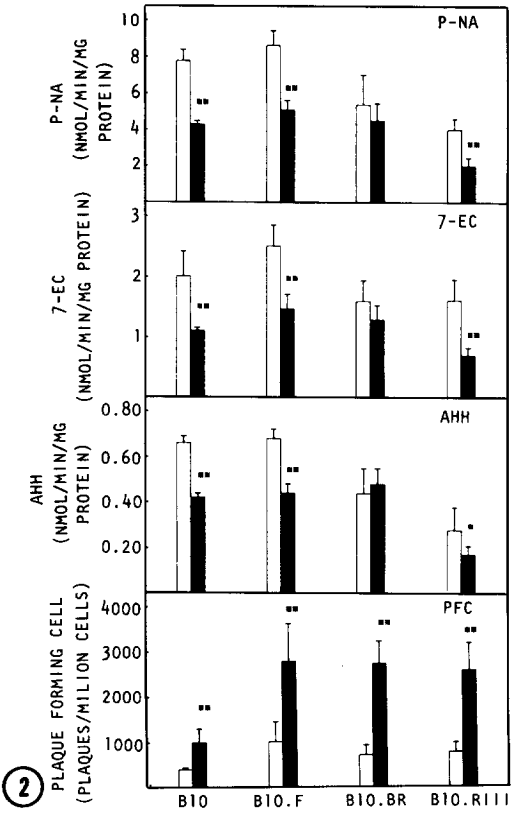


Figure 1. Increased activities of Types O,D, and O + D xanthine oxidases by treatment with poly IC (5 mg/kg, ip).

Bars show means and SDs. *: $P < 0.05$, **: $P < 0.01$ compared to basal levels. (□) = basal levels, (■) = following poly IC.

Figure 2. Enhancement of PFC and decrease of MFO activities by treatment with poly IC (5 mg/kg, ip).

Bars show means and SDs. *: $P < 0.05$, **: $P < 0.01$ compared to basal levels. (□) = basal levels, (■) = following poly IC.

Influence of Poly IC on PFC and P-450 Associated Enzymes

Poly IC treatment increased the PFC response (Figure 2). The extent of enhancement of PFC was 245 ± 73% of the control value in B10 (H-2b), 274 ± 83% in B10.F (H-2n), 385 ± 71% in B10.BR (H-2k) and 335 ± 79% in B10.RIII (H-2r), respectively. The degrees of enhancement of PFC were B10.BR (H-2k) = B10.RIII (H-2r) > B10.F (H-2n) = B10 (H-2b) in decreasing order (Duncan's multiple range test). There was a marginal yet significant inverse correlation ($P < 0.05$: $n = 19$) between the enhancement of PFC due to poly IC treatment and the

inducibility of total xanthine oxidase activity: as the inducibility of xanthine oxidase increased, the enhancement of PFC decreased ($r = -0.43$).

Poly IC decreased the P-450 content to 79 $\pm$ 7% of the control value in B10 and 73 $\pm$ 7% in B10.RIII mice (data not shown) while in other strains, no significant decrease was observed. Poly IC decreased the activities of AHH, 7-EC and P-NA by 35 to 43% in B10 (H-2b), B10.F (H-2n) and B10.RIII (H-2r), but did not influence those activities in B10.BR (H-2k) mice (Figure 2). A significant inverse correlation between the activity of AHH and the inducibility of total xanthine oxidase is shown in Figure 3 ($r = -0.71$, $P < 0.01$, $n = 19$). The suppressive effect of poly IC was saturated around 50% of AHH

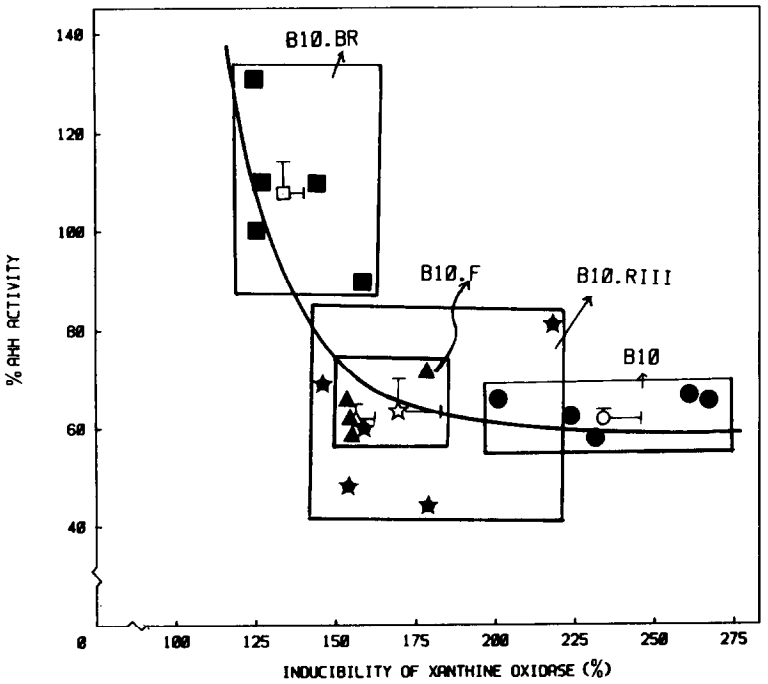


Figure 3. Relationship between % AHH activity and inducibility of total xanthine oxidase (XO + XD).

Percent of total xanthine oxidase (XO + XD), which is the ratio of individual animal's values over the control mean values, is depicted on the horizontal axis. Percent AHH, which is the ratio of individual animal's values over the control mean values, is shown on the vertical axis. (■), (▲), (★) and (■), show individual values. The open figures (□), (△), (☆) and (○) indicate the means and standard errors of the means of percent AHH activities and percent inducibilities of xanthine oxidase in B10.BR, B10.F, B10.RIII and B10 mice, respectively. The large rectangles mark the area where values of individual H-2 congenic mice scattered, and illustrate the segregation for different H-2 haplotype animals. The curve was fitted by using 2nd order polynomials.

suppression, which is considered to be the maximum suppression by poly IC or INFs treatment (15).

Effect of Poly IC Treatment on Heme Pool Associated Enzymes

Treatment with poly IC did not appear to change activities of ALA-S, tryptophan pyrrolase and catalase 24 hours after treatment in any of the mouse strains (data not shown).

DISCUSSION

It is known that poly IC, a potent IFN (α and β) inducer, and the IFNs *per se* induce xanthine oxidase (1). In the present study, we have demonstrated that H-2 haplotype has an influence on the inducibility of xanthine oxidase activity 24 hours following treatment with poly IC. Since the induction of xanthine oxidase is reported to reach maximum 24 hours after treatment (1), differences in xanthine oxidase activities can conceivably be due to differences in inducibility rather than differences in induction kinetics.

Since we did not measure the actual amounts of INFs, we cannot distinguish whether the H-2 haplotype has a direct influence on the inducibility of xanthine oxidase, or an indirect influence by means of an effect on the inducibility of IFNs. Thus one might postulate an H-2 linked gene concerned with xanthine oxidase levels. If the effect is indirect, the possibility of another IFN loci besides IF-1 ~ 4 can be suggested. This new locus should be close to or within the H-2 complex. An indirect effect appears to be more likely. Our speculation is based on evidence which indicate that induction of xanthine oxidase, after INF inducer treatments, occurs only when induction of INFs occurs and INF *per se* induces xanthine oxidase (1, 2).

It is known that poly IC or IFN *per se* decrease the activities of MFOs. In the present study we observed H-2 effects on these decreases (Figure 2). Since we could not detect any changes in activities of ALA-S, tryptophan pyrrolase or catalase 24 hours after treatment with poly IC in any of the congenic mice, genetic differences in heme synthesis cannot explain intra-strain differences in the decrease of MFO activities. In contrast, as shown clearly in Figure 3, decreases in AHH activities following poly IC showed a

significant correlation with increases in xanthine oxidase inducibility, which in turn correlates with the H-2 haplotype (Figure 1). Thus the apparent H-2 effect on the poly IC induced decreases in activities of P-450 dependent enzymes can be explained by the increases in xanthine oxidase. Therefore the present work is in line with the hypothesis that poly IC induced decreases in the activities of MFOs result from increased destruction of P-450 due to increased levels of active oxygen (2). Our postulated order of progression then becomes poly IC $\rightarrow$ IFN $\uparrow$ $\rightarrow$ xanthine oxidase $\uparrow$ $\rightarrow$ MFO $\downarrow$, and the H-2 influence is at the IFN level.

It is of interest that enhancement of the PFC response might correlate with the inducibility of xanthine oxidase ($r = -0.43$, $P < 0.05$). The effects of the IFNs on immune reactions are complex. The IFNs influence several immunocompetent cell types. In general, B cell activity is inhibited, T cell activity enhanced. In the present study we found that poly IC administration enhanced PFC in an H-2 related manner. This might be due to a combination of activation and suppressive effects on the immune system since both B and T cells are involved in the PFC response. Interferon induced suppression of B cell function may be partly mediated by oxygen metabolites (9). Thus, theoretically it is possible that induced xanthine oxidase plays a role in B cell suppression, thereby limiting the enhancement of PFC. We should obviously study the effects of IFNs on different immune competent cell populations to investigate the mechanisms of this phenomenon.

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