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MECHANISM OF HYBRID RESISTANCE

The Role of a Natural Antibody in Parental Bone Marrow Cell Rejection[1]

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Hybrid resistance (HR) to parental bone marrow growth is specifically directed against hemopoietic histocompatibility (Hh-1) Ag that are present in parental bone marrow cells (bmc). The mechanism of HR seems to be a multistep process. According to a model we proposed earlier, a T cell recognizes the Hh-1 Ag and stimulates a macrophage to secrete IFN-α/β (recognition phase). IFN-α/β activates a NK-like cell that specifically kills the parental bmc (effector phase). We have also described in a previous paper that serum from resistant F1 hybrids contains a humoral factor that seems to be involved in the effector phase of HR. In the present work, we study the role and the nature of this humoral factor. Our results show that this humoral factor: 1) is present in all resistant H-2D^b heterozygous F1 hybrids we have tested but not in nonresistant H-2D^b homozygous mice; 2) seems to recognize the Hh-1^b Ag because it is absorbed on bmc from Hh-1^b mice but not on bmc from Hh-1^d and Hh-1$^-$ mice; and 3) is an IgG1 Ig (natural antibody). These results could help us to explain the specificity of HR at the effector phase by supposing that this natural antibody recognize the Hh-1 Ag and enable NK-like cells to kill parental bmc cells in Hh-1 specific manner.

One of the unexplained phenomena in transplantation immunology is the rejection of parental bmc[3] by a lethally irradiated F1 hybrid (1) referred to as HR. HR to parental bmc is directed against Hh-1 Ag that are encoded by the Hh-1 genes located between the H-2S and H-2D regions (2); the most frequent allele (Hh-1^b) is always linked to the H-2D^b haplotype (3). Parental bmc rejection is a multistep process (4–6). In a first step, a CD5$^+$ CD4$^-$CD8$^-$CD3$^+$a-GM1$^-$ 5-fluorouracil-sensitive T cell in the F1 hybrid recognizes the Hh-1 Ag present on the parental bmc and stimulates a macrophage-like cell to secrete IFN-α/β (recognition phase). In the second step, IFN-α/β activates a NK cell that is thought to be the final effector cell (effector phase).

However, this model cannot explain the specificity of HR mainly in the effector phase. NK cells seem to be improbable mediators of specificity inasmuch as they do not have Ag-specific receptors (7). Moreover, the natural antibodies that are involved in the allogeneic resistance and could mediate specificity have not been detected in the case of HR either because they really do not play a role in parental bmc rejection or because there was not a suitable model that allowed their detection (8). Recently, Yankelevich et al. (9) have reported that the final effector cell of both allogeneic and hybrid resistance belongs to the T cell lineage and bears Ag-specific TCR. This observation could explain the specificity of HR since TCR$^+$ cells could theoretically specifically recognize the Hh-1 Ag.

In a previous paper (6) dealing with the mechanism of the specific abrogation of HR in F1 hybrids pretreated with parental spleen cells (pretreated F1 hybrids) (10–13), we have shown that serum from normal F1 mice contains a humoral factor that can restore HR when transferred into a nonresistant pretreated F1 hybrid; this humoral factor seems to be involved in the effector phase of HR. We were thus interested in developing a study on the nature and the role of this humoral factor in parental bmc rejection. Our results showed that this humoral factor: 1) is present in all resistant H-2D^b heterozygous hybrids; 2) can be absorbed on Hh-1^b bearing bmc; and 3) is an IgG1 isotype Ig.

MATERIALS AND METHODS

Animals. Specific pathogen-free C57BL/10 (B10), B10.HTG, B10.D2(R106) (R106), (C57BL/10 × DBA/2)F1 (B10D2F1), (C57BL/10 × C3H)F1 (B10C3F1), (C57BL/10 × NZW)F1 ((B10 × NZW)F1), and (C57BL/10 × B10.A)F1 ((B10 × B10.A)F1) mice were obtained from the breeding center of the Centre National de la Recherche Scientifique Orléans La Source, France. The hybrids (C57BL/10 × B10.D2(R106)F1 ((B10 × R106)F1), (C57BL/10 × B10.HTG)F1 ((B10 × HTG)F1), (C57BL/10 × B10.D2)F1) ((B10 × B10.D2)F1), and (B10.D2 × B10.HTG)F1 ((B10.D2 × HTG)F1) were bred in our own facilities. The genetic composition of all strains is listed in Table I.

Irradiation. A cesium-137 source delivering approximately 80 rad/min was used for the irradiation.

Pretreatment of B10D2F1 hybrids with B10 spleen cells. Normal B10D2F1 hybrids were injected i.v. with 5×10^7 parental B10 spleen cells 1 week before bmc grafting; we shall refer to these hybrids as B10-pretreated B10D2F1 mice.

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[3] Abbreviations used in this paper: bmc, bone marrow cells; HR, hybrid resistance; Hh-1, hemopoietic histocompatibility.

TABLE 1
Genetic composition of strains used

Strain	H-2 Haplotype				Hh-1
	K	IA	IE	D	
B10	b	b	—	b	Hh-1^b
B10.D2	d	d	d	d	Hh-1^d
B10.HTG	d	d	d	b	Hh-1^b
B10.D2 (R106)	b	b	—	d	Hh-1^d
B10.A	k	k	k	d	Hh-1^d
DBA/2	d	d	d	d	Hh-1$^-$
NZW	u	u	u	z	Hh-1^z
C3H	k	k	k	k	Hh-1$^-$

Preparation of cell suspensions for injection. bmc suspensions were prepared aseptically by removing the femurs and tibias from mice, clipping off the ends, and aspirating the marrow through a syringe fitted to a 23-gauge needle into HBSS. Spleen cell suspensions were prepared by aseptically removing the spleens and pressing them between two microscope slides. The spleen cell suspension was then filtered through gauze. In both cases, viable cells were counted using trypan blue exclusion, and the suspension adjusted to the appropriate concentration. The desired numbers of bmc and spleen cells were injected in 0.5 ml in HBSS or F1 serum as indicated in each Figure.

Fractionation of mouse sera on protein A-Sepharose column. The method used is a modification of the one described by Ey et al. (14). Briefly, a stepwise elution of the protein A-Sepharose CL4B (Pharmacia Fine Chemicals, Uppsala, Sweden) was used, consisting of the application of buffers with decreasing pH values (NaCl-Tris, pH 8.0; acetate buffers with pH 6.0, 5.5, and 4.0). All fractions were set up to the same starting serum volume. Purity of each fraction and protein concentration of each isotype (IgM, IgA, IgG1, IgG2a, and IgG2b) was estimated by means of radial immunodiffusion using specific antisera and mAb against relevant heavy chain. Concentration of IgG1 and IgG2 was also estimated as the basis of *A* at 280 nm with $E^{1\%}_{280} = 1.5$. IgG1 fraction was not contaminated by any other isotype of Ig, whereas in the IgG2 fraction there was a very slight contamination by IgG1 (<0.5%). IgG were not detected in the IgA + IgM fraction. The values for different Ig isotypes were as follows: IgG1 = 0.350 mg ± 0.05/ml, IgG2a = 1.5 mg ± 0.15/ml, IgG2b = 0.150 mg ± 0.05/ml, IgA = 0.650 ± 0.10/ml, and IgM = 0.680 mg ± 0.20/ml.

Absorption of B10D2F1 serum on bmc. Undiluted B10D2F1 serum was incubated with bmc (10^8/ml) from different mouse strains at 37°C. After 2 h of incubation, the cell suspension was centrifuged and serum recovered.

Experimental protocol. Normal or B10-pretreated B10D2F1 hybrids were irradiated at 950 rad and 6 h later grafted with 2×10^6 B10 bmc. On day 6 postgrafting, 2 μCi of ^{125}I-deoxyuridine were injected i.p. into the hybrids. The spleens were removed the next day, their radioactivity counted using a γ-counter, and the percentage of the injected radioactivity incorporated in the spleen calculated. Results are expressed as a percentage of the radiolabel incorporated in the spleens of B10 mice grafted with 2×10^6 syngeneic bmc (syngeneic control) calculated for each experiment independently. All experiments included ungrafted controls of the hemopoietic autorestoration of the recipients (^{125}I-deoxyuridine incorporation < 0.004%).

Statistics. Each Figure presents results of at least four independent experiments, as indicated in their legends. Significance of results was determined using Student's *t* test.

RESULTS

Transfer of B10D2F1 serum into B10-pretreated B10D2F1 hybrids restores HR. Normal and B10-pretreated B10D2F1 hybrids were injected with B10 bmc along with serum from normal or B10-pretreated B10D2F1 mice. B10 bmc proliferation measured 6 days later showed that HR, overcome in the B10-pretreated B10D2F1 recipients, was partially restored by the transfer of serum from normal B10D2F1 but not by the transfer of serum from B10-pretreated B10D2F1 mice (Fig. 1*A*). Moreover, B10-pretreated B10D2F1 hybrids rejected also B10 bmc that were preincubated with serum from normal B10D2F1 and washed twice before being grafted (Fig. 1*B*).

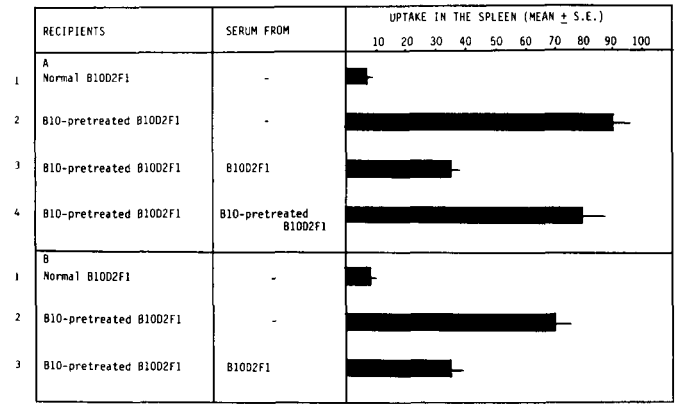

Figure 1. Partial restoration of HR in B10-pretreated B10D2F1 hybrids by transfer of serum from normal B10D2F1 mice. *A*, B10-pretreated B10D2F1 hybrids were grafted with B10 bmc suspended in serum from normal or B10-pretreated B10D2F1 mice. *B*, B10-pretreated B10D2F1 hybrids were grafted with B10 bmc cells incubated with HBSS or B10D2F1 serum for 2 h, washed twice, and resuspended in HBSS. Significant differences in ^{125}I-deoxyuridine incorporation were found for groups 2 and 1, and 2 and 3 ($p < 0.001$) for *A*; 2 and 1, and 2 and 3 ($p < 0.01$) for *B*. Composite of 12 (*A*) and 14 (*B*) independent experiments.

These results confirm our previous observations (6) that serum from resistant F1 hybrids restores HR in parental spleen cell-pretreated F1 mice, and strongly suggest that the humoral factor responsible for this effect acts by being absorbed on parental bmc.

Transfer of serum from different resistant F1 hybrids into B10-pretreated B10D2F1 mice restores HR. From results presented in Figure 1 as well as results published previously (6) arises the question as to whether the humoral factor is present in all hybrids expressing HR; in other words, whether the presence of humoral factor always correlates with the capacity of F1 hybrids in rejecting parental bmc. Anti-B10-parent HR is observed in all H-2D^b heterozygous hybrids (3). If the humoral factor is always necessary for B10 bmc rejection, then it will be produced by all H-2D^b heterozygous resistant mice. In the first series of experiments performed to study this hypothesis, B10-pretreated B10D2F1 mice were injected with B10 bmc along with serum from either resistant, B10D2F1, B10C3F1, (B10 × NZW)F1, and (B10 × B10.A)F1 hybrids, or from nonresistant B10 mice. In all these hybrids, the H-2 heterozygosity was extended to the K, I, and D regions. B10 bmc engraftment otherwise enhanced in B10-pretreated B10D2F1 recipients was inhibited by the transfer of serum from all H-2^b heterozygous F1 hybrids tested while it remained enhanced after transfer of serum from B10 mice (Fig. 2).

In the second series of experiments, we tested to determine whether HR can be restored by transfer of serum from F1 mice in which H-2 heterozygosity is limited to the H-2D region. Figure 3 shows that in B10-pretreated B10D2F1 hybrids, HR is partially restored by the transfer of serum from resistant H-2D^b heterozygous (B10.D2 × HTG)F1 and (B10 × R106)F1 mice; transfer of serum from nonresistant H-2D^b homozygous (B10 × HTG)F1 and B10 mice resulted in a very slight, nonsignificant inhibition of B10 bmc engraftment. Comparable slight and nonsignificant inhibition of parental bmc proliferation was also observed after transfer of serum from nonresistant parental spleen cell-pretreated F1 hybrids (6).

Taken together, these results prove that humoral factor involved in the expression of HR is present only in H-2D^b

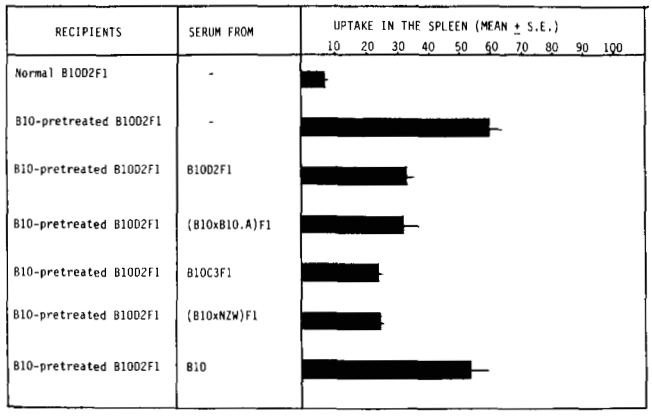

Figure 2. Partial restoration of HR in B10-pretreated B10D2F1 hybrids by transfer of serum from resistant H-2^b heterozygous F1 hybrids. B10-pretreated B10D2F1 hybrids were grafted with B10 bmc suspended in serum from normal B10D2F1 (B10 × B10.A)F1, B10C3F1, (B10 × NZW)F1, or B10 mice. Significant differences in ^{125}I-deoxyuridine incorporation were found for groups 2 and 1, 2 and 3, 2 and 4, 2 and 5, and 2 and 6 ($p < 0.01$). Composite of four independent experiments.

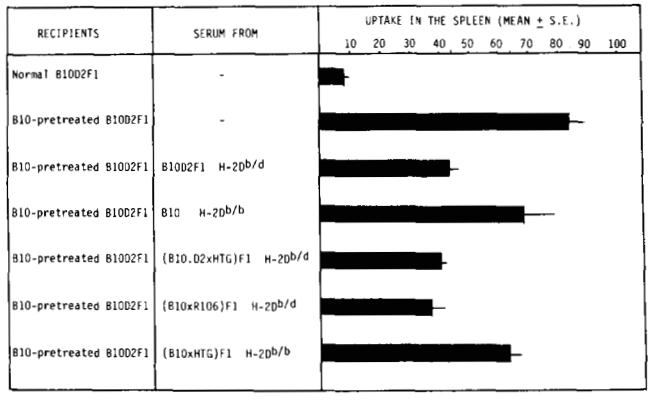

Figure 3. Partial restoration of HR in B10-pretreated B10D2F1 hybrids by transfer of serum from resistant H-$2D^b$ heterozygous F1 hybrids. B10-pretreated B10D2F1 hybrids were grafted with B10 bmc suspended in serum from B10D2F1, B10, (B10.D2 × HTG)F1, (B10 × R106)F1, or (B10 × HTG)F1 mice. Significant differences in ^{125}I-deoxyuridine incorporation were found for groups 2 and 1, 2 and 3, 2 and 5, and 2 and 6 ($p < 0.01$). Composite of four independent experiments.

heterozygous mice resistant to B10 bmc growth. Moreover, they suggest that the H-2D region controls the production of this factor since the humoral factor-producing and humoral factor-nonproducing mice differ only at H-2D region level.

B10D2F1 serum absorbed on H-2D$^{b/b}$ bmc cannot restore HR in B10-pretreated B10D2F1 hybrids. It is known that HR is specifically directed against Hh-1 Ag and that Hh-1^b Ag are always associated with the H-$2D^b$ haplotype (3). Moreover, in a previous paper we have shown that humoral factor is involved in the final effector phase of HR, probably by allowing NK-like cells to specifically kill grafted bmc (6). For this hypothesis to be true, the humoral factor should be able to recognize specifically the Hh-1^b Ag.

Therefore, B10-pretreated B10D2F1 hybrids were injected with B10 bmc along with B10D2F1 serum either untreated or absorbed on bmc from different strains of mice that either did or did not express Hh-1^b Ag. B10D2F1 serum inhibited B10 bmc growth when it was untreated or absorbed on bmc from H-$2D^{b/d}$/Hh-1^- ((B10D2F1 and (B10 × R106)F1) or from H-$2D^{d/d}$/Hh-1^d (R106) mice. In contrast, it lost the capacity to restore HR when it was absorbed on bmc from H-$2D^{b/b}$/Hh-1^b (B10, B10.HTG, and (B10 × HTG)F1) mice (Fig. 4).

These results show that H-$2D^{b/b}$/Hh-1^b cells absorb the humoral factor from the B10D2F1 serum and suggest that humoral factor can specifically recognize the Hh-1^b Ag.

Humoral factor belongs to the IgG family. To recognize specifically the Hh-1^b Ag, the humoral factor should be an Ig. Therefore, the B10D2F1 serum was fractionated on a protein A-Sepharose column and the different fractions obtained were tested for their capacity to restore HR in nonresistant B10-pretreated B10D2F1 hybrids. Results in Figure 5 show that IgG1 fractions restored HR, whereas IgG2 and IgG-free fractions did not. These results clearly demonstrate that the humoral factor we described is an IgG1 Ig.

DISCUSSION

Pretreatment of a F1 hybrid with parental spleen cells completely abrogates HR (11–14). This abrogation, attrib-

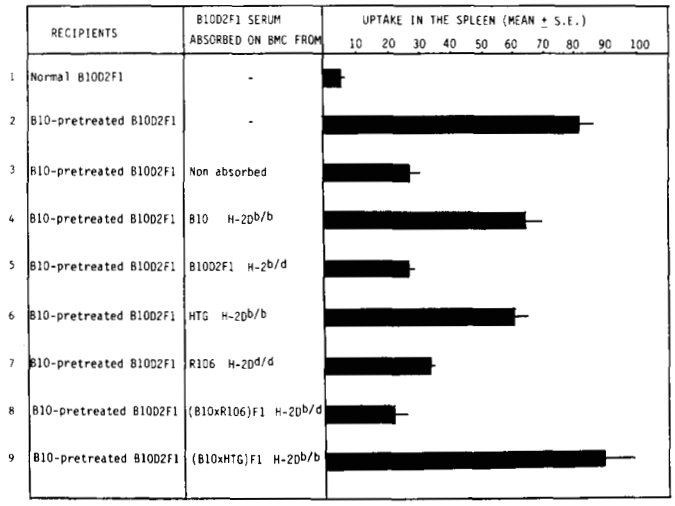

Figure 4. Inability of B10D2F1 serum absorbed in Hh-1^b bmc to restore HR in B10-pretreated B10D2F1 hybrids. B10-pretreated B10D2F1 mice were grafted with B10 bmc suspended in B10D2F1 serum nonabsorbed or absorbed on bmc from B10, B10D2F1, HTG, R106, (B10 × R106)F1, or (B10 × HTG)F1 mice. Conditions of absorption were described in *Materials and Methods*. Significant differences in ^{125}I-deoxyuridine incorporation were found for groups 2 and 1, 2 and 3, 2 and 5, 2 and 7, and 2 and 8 ($p < 0.01$). Composite of five independent experiments.

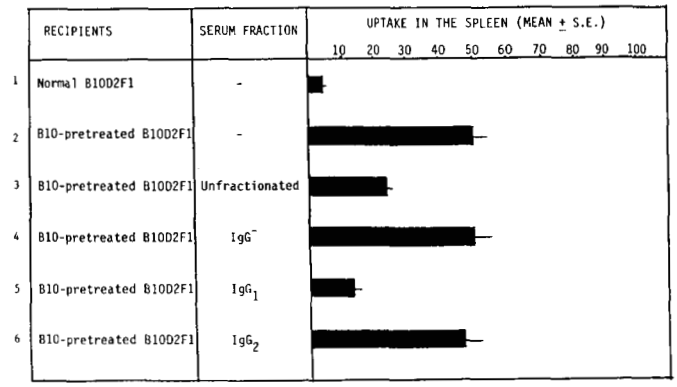

Figure 5. Partial restoration of HR in B10-pretreated B10D2F1 hybrids transferred with the IgG1 fraction of B10D2F1 serum. B10-pretreated B10D2F1 hybrids were grafted with B10 bmc suspended either in unfractionated B10D2F1 serum or in B10D2F1 serum fractionated on a protein A-Sepharose column (see *Materials and Methods*). Significant differences in ^{125}I-deoxyuridine incorporation were found for groups 2 and 1, 2 and 3, and 2 and 5 ($p < 0.01$). Composite of four independent experiments.

utable to a specific Hh-1 Ag tolerization of the pretreated F1 hybrids (12), results from: 1) the induction of a $CD5^+CD3^+CD4^+CD8^-$ cell that suppresses the T cell involved in the initial recognition phase of HR (6, 11); 2) the graft vs host associated suppression of the NK-like effector cell for HR (13, 15); and 3) the consumption of a humoral factor that seems to be involved in the final effector phase of HR (6). The humoral factor was demonstrated in experiments in which the injection of normal B6C3F1 serum into a nonresistant B6-pretreated B6C3F1 mouse partially restored HR, whereas the injection of serum from B6-pretreated B6C3F1 hybrid did not. The optimal activity of this factor depends on the presence of functional NK cells since restoration of HR was complete only when the injection of F1 serum was associated with transfer of F1 spleen cells and daily administration of IFN-α/β (6).

In this work we have studied the nature and the role of the humoral factor in the expression of HR. The first series of results showed that this humoral factor is detectable in the serum of all of the resistant F1 hybrids we have tested. In fact, the serum from resistant (B10 × C3H)F1, (B10 × NZW)F1, B10D2F1, and (B10 × B10.A)F1 mice restored HR when injected into nonresistant B10-pretreated B10D2F1 hybrids, whereas the serum from nonresistant B10 and (B10 × HTG)F1 mice did not. It is known that rejection of $H\text{-}2^b$ parental bmc requires the specific recognition of $Hh\text{-}1^b$ Ag always associated with the $H\text{-}2D^b$ homozygous haplotype; $H\text{-}2D^b$ heterozygous hybrids are $Hh\text{-}1^-$ and therefore resist $Hh\text{-}1^b$ bmc growth by developing an anti-$Hh\text{-}1^b$ reaction. However, this reaction does not require a prior immunization of the hybrids with $Hh\text{-}1^b$ Ag; that means that resistant hybrids must possess a natural mechanism making them able to recognize $Hh\text{-}1^b$ Ag and to reject $Hh\text{-}1^b$ parental bmc. Our results show that $H\text{-}2D^b$ heterozygous hybrids produce a humoral factor that is involved in HR, whereas $H\text{-}2D^b$ homozygous mice do not. Thus, the serum from $H\text{-}2D^b$ heterozygous B10D2F1, (B10.D2 × HTG)F1, and (B10 × R106)F1 mice restored HR when injected into B10-pretreated B10D2F1 recipients, whereas serum from $H\text{-}2D^b$ homozygous (B10 × HTG)F1 mice did not. It should be emphasized that B10D2F1 and (B10 × HTG)F1 hybrids genetically differ only in the H-2D region.

These results taken together show that there is a striking correlation between the presence of this humoral factor and the ability of F1 hybrids to resist $Hh\text{-}1^b$ parental bone marrow growth. Moreover, production of humoral factor seems to be controlled by the H-2D region because it always requires $H\text{-}2D^b$ heterozygosity.

HR is defined as an $Hh\text{-}1^b$ specific phenomenon. Daley and Nakamura (16) have clearly demonstrated that $H\text{-}2D^b$ homozygous/$Hh\text{-}1^b$ tumoral cells can compete for HR, whereas $H\text{-}2D^b$ heterozygous/$Hh\text{-}1^-$ cells cannot even if they are targets of in vitro NK activity (16); by using $H\text{-}2D^-/Hh\text{-}1^b$ mutants of the $H\text{-}2D^b$ homozygous/$Hh\text{-}1^b$ RBL-5 cell line, Milisauskas et al. (17) have proven that competition is due to the $Hh\text{-}1^b$ rather than to the class I $H\text{-}2D^b$ Ag. However, these observations raise a question: how does the NK cell, which normally does not bear a receptor that is specific for an Ag, specifically recognize $Hh\text{-}1^b$ Ag during the effector phase of HR? To answer this question, Warner and Dennert (8) proposed a hypothesis according to which specificity of the effector phase of bmc rejection is ensured by: 1) the direct recognition of the $Hh\text{-}1^b$ Ag by the "receptor" of the NK cell; and 2) the presence in the resistant mice of natural antibodies that could recognize $Hh\text{-}1^b$ Ag and kill grafted cells in an antibody-dependent cell-mediated cytotoxicity-like manner. Recent results of Yankelevich et al. (9), showing that HR effector cells bear the CD3/TCR complex, could explain the direct $Hh\text{-}1^b$ Ag recognition by the HR effector cells. On the other hand, the enhancement of allogeneic and semiallogeneic bmc rejection by antibodies was also clearly demonstrated (8, 18). For example, the nonresistant mice rejected allogeneic bmc that had been preincubated with anti-donor class I antibodies (18). Moreover, serum from resistant mice injected into nonresistant strains rendered them able to reject allogeneic bmc (8); this latter phenomenon was attributed to natural antibodies involved in allogeneic resistance. However, in the case of HR, although antiparent class I antibodies enhanced HR, the presence of the natural antibodies has not been proven (8), probably because of the lack of an appropriate experimental model making it possible to detect them. Results in the present paper show that these natural antibodies do exist in the serum of resistant F1 hybrids. In fact, the humoral factor we describe belongs to the IgG1 family; transfer of the IgG1 fraction of B10D2F1 serum restored HR in B10-pretreated B10D2F1 hybrids, whereas transfer of the IgG2 fraction or the fraction containing IgM, IgA, and other serum components did not. Moreover, IgG1 natural antibodies seem to specifically recognize $Hh\text{-}1^b$ Ag; incubation of B10D2F1 serum with $Hh\text{-}1^b$ B10, B10.HTG, or (B10 × HTG)F1 bmc rendered it unable to restore HR, whereas incubation with $Hh\text{-}1^-$ B10D2F1 or (B10 × R106)F1 bmc did not. It must be noted that B10 and (B10 × HTG)F1 differ from (B10 × R106)F1 and B10D2F1 mice only with respect to the H-2D/Hh-1 region.

However, our results raise two important questions: 1) how do these $Hh\text{-}1^b$ specific antibodies contribute to the killing of the grafted parental bmc; and 2) how great is their importance in the mechanism of HR? Warner and Dennert (8) have shown that antibodies involved in allogeneic resistance can kill donor H-2 compatible tumor cells in an antibody-dependent cell-mediated cytotoxicity manner. Multiple experiments performed in our laboratory to detect either a cellular or a complement-mediated antibody-dependent cytotoxicity gave irreproducible results and do not allow us to suggest that anti-$Hh\text{-}1^b$ antibodies in the serum of B10D2F1 mice act by cellular or complement-mediated killing. However, the fact that cells must be present to obtain an optimal effect from these antibodies in vivo suggests that the mechanism of their action is cell-dependent (6).

Antiparent class I antibodies can enhance HR, but parental bmc rejection can occur even in their absence (18). These results taken together with the fact that the HR-involved natural antibodies have never been detected led Yankelevich et al (9) to propose that the effector phase of HR is exclusively mediated by the NK $CD3^+$ TCR^+ cell. Our results, however, do not agree with this conclusion. This is first because HR-involved natural antibodies do exist in the serum of resistant F1 hybrid, and second, because these antibodies seem to play an important role in the parental bmc rejection. Indeed, results presented in a previous paper (6) have shown that restoration of

HR in B6-pretreated B6C3F1 hybrids occurs only when B6C3F1 serum is injected along with B6 bmc; transfer of B6C3F1 spleen cells, even in association with daily injection of INF-α/β, does not result in restoration of HR. These results suggested that in our experimental model the HR cannot be expressed in the absence of natural antibodies.

In conclusion, our data show that anti-Hh-1^b natural antibodies are involved in an antibody-mediated mechanism of parental bone marrow rejection. However, they cannot exclude the coexistence of another antibody-independent, cell-mediated mechanism suggested by others (9).

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REFERENCES

1. **Cudkowicz, G., and M. Bennett.** 1971. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F1 hybrid mice. *J. Exp. Med. 134:1513.*
2. **Daley, P., J. M. Wroblewski, S. G. Kaminsky, and I. Nakamura.** 1987. Genetic control of the target structure recognized in hybrid resistance. *Immunogenetics 26:21.*
3. **Cudkowicz, G., and I. Nakamura.** 1983. Genetics of the murine hemopoeitic histocompatibility system. An overview. *Transplant. Proc. 15:2058.*
4. **Kosmatopoulos, K., A. Bravo Cuellar, J. Wachowiak, and S. Orbach-Arbouys.** 1987. Inhibition of hybrid resistance by 5-fluorouracil. Destruction of a Thy-1^+Lyt-1^+2^- spleen cell implicated in the expression of hybrid resistance. *Transplantation 44:202.*
5. **Affifi, M. S., V. Kumar, and M. Bennett.** 1985. Stimulation of genetic resistance to marrow graft in mice by interferon-α/β. *J. Immunol. 134:3739.*
6. **Cibotti, R., E. Churaqui, I. Bolonakis-Tsilivakos, D. Scott Algara, O. Halle-Pannenko, and K. Kosmatopoulos.** 1989. Specific inhibition of hybrid resistance in F1 hybrid mice pretreated with parent-strain spleen cells. III. Study of the mechanism. *J. Immunol. 143:3484.*
7. **Dennert, G., G. Vogeeswaren, and S. Yamagata.** 1981. Cloned cell lines with natural killer activity: specificity function and cell markers. *J. Exp. Med. 158:545.*
8. **Warner, F. J., and G. Dennert.** 1985. Bone marrow graft rejection is a function of antibody-directed natural killer cells. *J. Exp. Med. 161:563.*
9. **Yankelevich, B., C. Knobloch, M. Nowicki, and G. Dennert.** 1989. A novel cell type responsible for marrow graft rejection in mice. T cells with NK phenotype cause acute rejection of marrow graft. *J. Immunol. 142:3223.*
10. **Lotzova, E.** 1977. Induction of unresponsiveness to bone marrow grafts. *J. Immunol. 119:543.*
11. **Kosmatopoulos, K., and S. Orbach-Arbouys.** 1987. Specific inhibition of hybrid resistance in F1 hybrid mice pretreated with parent-strain spleen cells. I. Induction of a nylon-adherent Thy-1^+Lyt-1^+2^- suppressor cell. *J. Immunol. 137:3109.*
12. **Kosmatopoulos, K., D. Scott Algara, and O. Halle-Pannenko.** 1988. Specific inhibition of hybrid resistance in F1 hybrid mice pretreated with parent strain spleen cells. II. Evidence for a Hh-1 specific tolerization. *J. Immunol. 141:3285.*
13. **Hakim, T. F., and G. M. Shearer.** 1986. Abrogation of hybrid resistance to bone marrow engraftment by graft-versus-host induced immune deficiency. *J. Immunol. 137:3109.*
14. **Ey, S. J., Prowse, C. R. Jenkin.** 1978. Isolation of pure IgG_1, IgG_{2a} and IgG_{2b} immunoglobulin from mouse serum using protein A-Sepharose. *Immunochemistry 15:429.*
15. **Knobloch, C., and G. Dennert.** 1988. Loss of F1 hybrid resistance to bone marrow graft after injection of parental lymphocytes. Demonstration of parental anti F1 killer cells and general immunosuppression in the host. *Transplantation 45:175.*
16. **Daley, J. P., and I. Nakamura.** 1984. Natural resistance of lethally irradiated F1 hybrid mice to parental marrow grafts is a function of H-2/Hh restricted effectors. *J. Exp. Med. 159:1132.*
17. **Milisauskas, V. K., S. G. Kaminsky, and I. Nakamura.** 1987. Class I H-$2D^b$ determinants are not involved in hybrid resistance to parental H-2^b/Hh-1^b bone marrow allografts. *Eur. J. Immunol. 17:1043.*
18. **Dennert, G., C. G. Anderson, and J. Warner.** 1986. Induction of bone marrow allograft rejection and hybrid resistance in nonresponder recipients by antibody: is there evidence for a dual receptor interaction in acute marrow graft rejection? *J. Immunol. 136:3981.*